# SHORT COMMUNICATION

# Effects of supplemental xylanase on *in vitro* disappearance of dry matter in feed ingredients for swine

## *Efectos de la suplementación con xilanasa sobre la desaparición in vitro de materia seca en ingredientes de piensos para cerdos*

## *Efeitos da xilanase suplementar no desaparecimento in vitro de matéria seca em ingredientes de rações para suínos*

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***To cite this article:***
Jo H, Sung JY, Kim BG. Effects of supplemental xylanase on *in vitro* disappearance of dry matter in feed ingredients for swine. Rev Colomb Cienc Pecu 2021; 34(4): 316–323. DOI: https://doi.org/10.17533/udea.rccp.v34n4a08

**Abstract**

**Background:** Alternative feed ingredients are widely used in swine diets to lower feed costs, but these ingredients contain a large quantity of non-starch polysaccharides. Supplemental xylanase is known to break down non-starch polysaccharides. However, the effects of exogenous xylanase from *Bacillus subtilis* on various feed ingredients have rarely been compared. **Objective:** To evaluate the effects of supplemental xylanase on *in vitro* disappearance of dry matter (DM) in various feed ingredients for pigs. **Methods:** Nine feed ingredients were used to measure *in vitro* ileal disappearance and *in vitro* total tract disappearance of DM. Each ground ingredient was supplemented with either supplemental xylanase (9,000 U/g) or cornstarch at 1.0%. **Results:** Supplemental xylanase increased *in vitro* ileal disappearance of DM in wheat, barley, wheat flour, and wheat bran ($p<0.05$). The *in vitro* total tract disappearance of DM for barley and wheat bran increased with xylanase addition ($p<0.05$). **Conclusion:** Exogenous xylanase could increase *in vitro* ileal DM disappearance in barley, wheat, wheat flour, and wheat bran, but did not affect *in vitro* total tract DM disappearance in wheat and wheat flour.

**Keywords:** *dry matter digestibility; enzyme; feedstuffs; ileal digestibility; in vitro disappearance; non-starch polysaccharides; pigs; total tract digestibility; xylanase.*

*Received: August 29, 2019; accepted: January 28, 2021*

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**Resumen**

**Antecedentes:** Los ingredientes alternativos se utilizan ampliamente en las dietas porcinas para reducir los costos del pienso, pero estos ingredientes contienen una gran cantidad de polisacáridos no-amiláceos. Se sabe que la xilanasa suplementaria descompone los polisacáridos diferentes al almidón. Sin embargo, rara vez se han comparado los efectos de la xilanasa exógena de Bacillus subtilis en algunos ingredientes del alimento. **Objetivo:** Evaluar los efectos de la xilanasa suplementaria sobre la desaparición *in vitro* de la materia seca (MS) en varios ingredientes alimentarios para cerdos. **Métodos:** Se utilizaron nueve ingredientes del alimento para medir la desaparición ileal *in vitro* y la desaparición del tracto total *in vitro* de MS. Cada ingrediente molido se complementó con xilanasa suplementaria (9,000 U/g) o almidón de maíz al 1,0%. **Resultados:** La xilanasa suplementaria aumentó la desaparición ileal *in vitro* de MS en trigo, cebada, harina de trigo y salvado de trigo ($p<0,05$). La desaparición de tracto total de MS *in vitro* para la cebada y el salvado de trigo aumentó con la adición de xilanasa ($p<0,05$). **Conclusión:** La xilanasa exógena podría aumentar la desaparición de la MS ileal *in vitro* en cebada, trigo, harina de trigo y salvado de trigo, pero no afecta la desaparición de la MS en tracto total *in vitro* del trigo y la harina de trigo.

**Palabras clave:** *alimentos; cerdos; digestibilidad de tracto total; digestibilidad de la materia seca; digestibilidad ileal; desaparición in vitro; enzima; polisacáridos no almidonáceos; xilanasa.*

**Resumo**

**Antecedentes:** Ingredientes alternativos para rações são amplamente usados em dietas para suínos para reduzir os custos da alimentação, mas esses ingredientes contêm uma grande quantidade de polissacarídeos não amiláceos. A xilanase suplementar é conhecida por quebrar polissacarídeos não amiláceos. No entanto, os efeitos da xilanase exógena de Bacillus subtilis em vários ingredientes da ração raramente foram comparados. **Objetivo:** Avaliar os efeitos da xilanase suplementar no desaparecimento *in vitro* da matéria seca (MS) em vários ingredientesde rações para suínos. **Métodos:** Nove ingredientes da ração foram usados para medir o desaparecimento ileal *in vitro* e o desaparecimento de MS *in vitro* do trato total. Cada ingrediente moído foi suplementado com xilanase suplementar (9.000 U/g) ou amido de milhoa 1,0%. **Resultados:** A xilanase suplementar aumentou o desaparecimento ileal *in vitro* de MS em trigo, cevada, farinha de trigo e farelo de trigo ($p<0,05$). O desaparecimento *in vitro* de MS do trato total para cevada e farelo de trigo aumentou com a adição de xilanase ($p<0,05$). **Conclusão:** A xilanase exógena pode aumentar o desaparecimento *in vitro* da MS ileal em cevada, trigo, farinha de trigo e farelo de trigo, mas não afetou o desaparecimento *in vitro* do trato total da MS no trigo e na farinha de trigo.

**Palavras-chave**: *alimentos para animais; digestibilidade da matéria seca; digestibilidade ileal; digestibilidade do trato total; enzima; desaparecimento in vitro; polissacarídeos sem amido; porcos; xilanase.*

## Introduction

The price of conventional feed ingredients used as energy and protein sources for swine diets sharply fluctuates. Thus, the use of alternative feed ingredients such as copra meal, palm kernel expellers, and wheat distillers dried grains with solubles (DDGS) is inevitable to lower feed costs. However, alternative feed ingredients are generally high in non-starch polysaccharides (NSP) that act as anti-nutritional factors on feed energy values. Therefore, the inclusion of exogenous enzymes in swine diets is a common strategy to increase energy values by improving utilization of NSP as well as other energy-yielding nutrients in pigs.

Arabinoxylan, consisting of arabinose and xylose (Saulnier *et al.*, 2012), is the most common NSP found in cereal grains such as corn, wheat, and barley (Masey O'Neill *et al.*, 2014). Supplemental xylanase has been reported to break down NSP, particularly arabinoxylan, in monogastric animals (Kiarie *et al.*, 2013). Recently, a xylanase product (Nutrase Xyla®, Nutrex, Lille, Belgium) containing endo-1,4-β xylanase (9,000 U/g) originating from *Bacillus subtilis* has been reported to increase nutrient digestibility of a corn-soybean meal-based diet, and subsequently, to improve growth performance of growing pigs (Lee *et al.*, 2018). The effect of an enzyme is largely dependent on the substrate, and thus, the ingredient composition is one of the most important factors for the inclusion of supplemental enzymes in swine diets. However, the effects of exogenous xylanase from *Bacillus subtilis* on various feed ingredients have rarely been compared. *In vitro* assays have been used to evaluate the effect of supplemental enzymes on nutrient digestibility because these assays are less expensive and laborious compared with *in vivo* assays (Kong *et al.*, 2015; Ha *et al.*, 2020).

Therefore, the objective of this study was to evaluate the effects of supplemental xylanase on *in vitro* ileal disappearance (IVID) and *in vitro* total tract disappearance (IVTTD) of dry matter (DM) in various feed ingredients.

## Materials and methods

### *Enzyme and sample preparation*

A xylanase product (Nutrase Xyla®, Nutrex, Lille, Belgium) used in the present work originated from *Bacillus subtilis* and contained endo-1,4-β xylanase (9,000 U/g). The xylanase product was provided by Morning Bio Inc. (Cheonan, Republic of Korea). Nine feed ingredients were used to measure IVID and IVTTD of DM with or without supplemental xylanase. The ingredients used were corn, wheat, barley, wheat flour, copra meal, palm kernel expellers, corn DDGS, wheat DDGS, and wheat bran. Each ingredient was ground to pass a 1-mm screen (Cyclotech 1093; Foss Tecator AB, Höganäs, Sweden) and divided into two groups. Each group was supplemented with either cornstarch or xylanase at 1.0% resulting in 90 U of xylanase/g of each ingredient. As the current study was a pilot study for an *in vivo* study, the inclusion rate of xylanase was set to be high to determine whether DM disappearance was increased or not by supplemental xylanase, and subsequently, to screen potential feed ingredients for xylanase.

### *In vitro procedure*

The IVID procedure was a two-step enzymatic degradation that simulates digestion in the stomach and small intestine of the pig (Boisen and Fernández, 1995). In the first step, 1 g of ingredient sample was transferred into 100 mL conical flask and then 25 mL of sodium phosphate buffer solution (0.1 *M*, pH 6.0), and 10 mL of HCl (0.2 *M*, pH 0.7) were added. To simulate digestion conditions in the stomach, the pH was adjusted to 2.0 using 1 *M* HCl or NaOH solution, and 1 mL of freshly prepared pepsin solution (10 mg/mL; ≥250 U/mg solid, P7000, Pepsin from porcine gastric mucosa, Sigma-Aldrich, St. Louis, MO, USA) was added to the flask. To avoid bacterial fermentation, 0.5 mL of chloramphenicol (C0378, Chloramphenicol, Sigma-Aldrich, St. Louis, MO, USA) solution (5 g/L of ethanol) was also added. The flasks were closed with a silicon stopper and incubated in a shaking incubator at 39 °C for 6 h. After incubation, the second step simulated the

digestion in the small intestine of pigs. Firstly, 10 mL of phosphate buffer solution (0.2 *M*, pH 6.8), and 5 mL of 0.6 *M* NaOH solution were added to the flasks. Then the pH was adjusted to 6.8 using 1 *M* HCl or NaOH solution, and 1 mL of freshly prepared pancreatin solution (50 mg/mL; 4 × USP, P1750, Pancreatin from porcine pancreas, Sigma-Aldrich, St. Louis, MO, USA) was added. Then, the flasks were incubated in a shaking incubator at 39 °C for 18 h. After incubation, 5 mL of 20% sulfosalicylic acid solution was added and samples were left for 30 min at room temperature to precipitate the indigestible protein. After 30 min of precipitation, undigested samples were filtered through pre-dried and weighed glass filter crucibles (Filter Crucibles CFE Por. 2, Robu, Hattert, Germany) containing 400 mg of celite as filter aid using the Fibertec System (Fibertec System 1021 Cold Extractor, Tecator, Höganäs, Sweden). The flasks were rinsed twice with 1% sulfosalicylic acid solution, and 10 mL of 95% ethanol and 99.5% acetone were added twice to the glass filter crucibles. Glass filter crucibles with undigested samples were dried at 80 °C for 24 h. After 1 h of cooling in a desiccator, glass filter crucibles were weighed.

The IVTTD procedure used was a three-step enzymatic degradation that simulates the digestion in the stomach, the small intestine, and the large intestine of pigs (Boisen and Fernández, 1997). The first and second steps were similar to the IVID procedure except the weight of the sample, concentration of the enzymes, and incubation time. For IVTTD, 0.5 g of sample was used, and the concentrations of pepsin and pancreatic solutions were increased to 25 and 100 mg/mL, respectively, while the incubation times were reduced to 2 and 4 h, respectively. In the third step, 10 mL of 0.2 *M* EDTA solution was added to the flasks. The pH was then adjusted to 4.8 by adding 30% acetic acid or 1 *M* NaOH. Samples were supplemented with 0.5 mL of multi-enzyme (V2010, Viscozyme® L, Sigma-Aldrich, St. Louis, MO, USA) as a substitute for microbial enzymes, and incubated in a shaking incubator for 18 h at 39 °C. After incubation, the undigested residues were collected and glass filter crucibles with undigested residues were dried at 130 °C for 6 h. The number of replications per each treatment was 3 (one replication per batch).

*Chemical analysis*

Ingredients were analyzed for DM (Ahn *et al.*, 2014), crude protein (method 990.03; AOAC, 2005), ether extract (method 2003.05; AOAC, 2005), neutral detergent fiber (method 2002.04; AOAC, 2005), acid detergent fiber (method 973.18; AOAC, 2005), and ash (method 942.05; AOAC, 2005).

*Calculation and statistical analysis*

The IVID and IVTTD of DM were calculated with the following equations, respectively (Ha *et al.*, 2020):

$$\text{IVID or IVTTD of DM (\%)} = [(DM_{TI} - DM_{UR}) \div DM_{TI}] \times 100,$$

where $DM_{TI}$ and $DM_{UR}$ are the weight of DM concentration in the test ingredient and undigested residues, respectively.

Data were analyzed by the GLM procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC, USA; 2012). The model included xylanase addition as a fixed variable (Seo *et al.*, 2018). The experimental unit was the test flask. Statistical significance of treatment effects was declared at $p<0.05$.

## Results

Neutral detergent fiber of test ingredients ranged from 10.4 to 62.0% (Table 1).

Xylanase addition increased the IVID of DM in wheat, barley, wheat flour, and wheat bran compared with the non-supplemental xylanase group ($p<0.05$; Table 2).

The IVTTD of DM in barley and wheat bran increased by xylanase addition ($p<0.05$; Table 3). In contrast, supplemental xylanase did not affect IVID and IVTTD of DM in the other ingredients.

**Table 1.** Analyzed composition of test ingredients (as-is basis).

| Item, % | Corn | Wheat | Barley | Wheat flour | Copra meal | Palm kernel expellers | Corn DDGS | Wheat DDGS | Wheat bran |
|---|---|---|---|---|---|---|---|---|---|
| Dry matter | 87.7 | 89.8 | 89.8 | 87.9 | 89.6 | 90.4 | 89.6 | 89.9 | 88.9 |
| Crude protein | 8.1 | 11.1 | 11.0 | 15.7 | 22.3 | 15.3 | 26.9 | 35.8 | 14.6 |
| Ether extract | 3.9 | 2.1 | 2.8 | 3.7 | 1.9 | 6.5 | 9.1 | 7.8 | 3.9 |
| NDF | 11.0 | 10.4 | 16.1 | 19.7 | 54.5 | 62.0 | 31.9 | 29.4 | 42.6 |
| ADF | 2.4 | 2.7 | 5.5 | 4.0 | 30.1 | 42.0 | 10.4 | 11.1 | 13.6 |
| Ash | 1.7 | 1.4 | 2.4 | 2.0 | 6.9 | 4.5 | 5.2 | 4.6 | 4.8 |

DDGS = distillers dried grains with solubles; NDF = neutral detergent fiber; ADF = acid detergent fiber.

**Table 2.** *In vitro* ileal disappearance of dry matter in feed ingredients with or without xylanase addition[1].

| Item | Control | Xylanase | SEM | p-value |
|---|---|---|---|---|
| Corn | 82.0 | 83.0 | 0.7 | 0.372 |
| Wheat | 87.1 | 90.0 | 0.4 | 0.005 |
| Barley | 78.8 | 80.5 | 0.4 | 0.049 |
| Wheat flour | 84.1 | 86.6 | 0.4 | 0.014 |
| Copra meal | 48.0 | 48.3 | 0.3 | 0.649 |
| Palm kernel expellers | 28.2 | 29.5 | 0.6 | 0.211 |
| Corn distillers dried grain with solubles | 54.8 | 56.9 | 0.9 | 0.183 |
| Wheat distillers dried grain with solubles | 63.8 | 63.6 | 0.4 | 0.676 |
| Wheat bran | 49.5 | 53.6 | 0.6 | 0.007 |

[1]Each least squares mean represents 3 observations; SEM = standard error of the mean.

**Table 3.** *In vitro* total tract disappearance of dry matter in feed ingredients with or without xylanase addition[1].

| Item | Control | Xylanase | SEM | p-value |
|---|---|---|---|---|
| Corn | 84.8 | 86.9 | 0.7 | 0.112 |
| Wheat | 91.0 | 92.0 | 1.0 | 0.483 |
| Barley | 85.2 | 85.9 | 0.1 | 0.024 |
| Wheat flour | 91.4 | 91.8 | 0.4 | 0.590 |
| Copra meal | 73.6 | 72.9 | 0.5 | 0.355 |
| Palm kernel expellers | 39.3 | 40.9 | 0.8 | 0.222 |
| Corn distillers dried grain with solubles | 66.4 | 66.3 | 0.8 | 0.944 |
| Wheat distillers dried grain with solubles | 75.0 | 76.0 | 0.8 | 0.453 |
| Wheat bran | 60.7 | 63.4 | 0.5 | 0.016 |

[1]Each least squares mean represents 3 observations; SEM = standard error of the mean.

## Discussion

In the present study, chemical composition of the test ingredients was within the range of values reported in the literature (Stein *et al.*, 2016; Son *et al.*, 2019). The *in vitro* DM disappearance procedure has been used to estimate the *in vivo* digestibility of energy and nutrients in feed ingredients (Park *et al.*, 2012) and to determine the efficacy of exogenous enzyme complexes (Park *et al.*, 2016; Ha *et al.*, 2020). The IVID and IVTTD of DM in test ingredients in the present work were within the range of reported values (Boisen and Fernández, 1995, 1997; Kong *et al.*, 2015; Park *et al.*, 2016). In previous studies, supplemental xylanase improved ileal and total tract digestibility of energy in pigs fed diets containing 30% wheat millrun or 30% wheat screening (Nortey *et al.*, 2007), but not in pigs fed diets mainly composed of wheat and wheat DDGS (Widyaratne *et al.*, 2009). The increased energy digestibility by xylanase was likely due to the hydrolysis of wheat arabinoxylans in the wheat millrun and wheat screening. As NSP are concentrated during the distillation process, corn DDGS and wheat DDGS contain relatively large amounts of NSP. Thus, dramatic effects of xylanase on digestibility of distillers dried grains were anticipated. In the present work, however, xylanase did not improve IVID or IVTTD of DM in corn DDGS and wheat DDGS. This result is likely due to changes or degradation of NSP in corn and wheat during the fermentation and drying processes that may result in xylanase being ineffective (Widyaratne *et al.*, 2009). Similarly, no effect of xylanase was found in IVID and IVTTD of copra meal and palm kernel expellers, and this may be partially explained by the low concentration of arabinoxylan (approximately 3%) in these ingredients, which is a main substrate for xylanase (Alshelmani *et al.*, 2014).

In the present study, IVTTD was greater than IVID in test ingredients regardless of xylanase addition. The greater IVTTD value is likely to be attributed to additional digestion by multi-enzymes in the step 3, which mimics digestion in the large intestine. While IVID of DM in wheat and wheat flour was increased by supplemental xylanase, IVTTD of DM in these ingredients was not affected by xylanase. This may be explained by masking effects of Viscozyme® containing arabanase, beta-glucanase, hemicellulase, and xylanase during the third step of the IVTTD procedure (Kong *et al.*, 2015). On the other hand, both IVID and IVTTD of DM were increased by supplemental xylanase in barley and wheat bran. The significant effect of xylanase on DM disappearance in wheat bran can be explained by the highest concentrations of arabinoxylan in wheat bran (23.2%, Bach Knudsen, 2014) among the ingredients tested in the present work. In addition, exogenous xylanase increased the energy digestibility of a diet containing mainly wheat and wheat bran (Dong *et al.* 2018). However, the reason for the significant effect of xylanase on DM disappearance in barley is unclear. But, the increment of ileal DM disappearance by xylanase was less (1.7 vs. 4.1% units) in barley than in wheat bran. This result may be partially attributed to the lower concentration of arabinoxylan in barley (8.4%) than that in wheat bran (23.2%; Bach Knudsen, 2014).

In conclusion, exogenous xylanase could increase IVID of DM in wheat, barley, wheat flour, and wheat bran, but no effect on IVTTD in wheat and wheat flour was found. Further research is warranted to determine the effects of supplemental xylanase on other feed ingredients and confirm the effect of xylanase in experiments with animals.

## Declarations

*Acknowledgements*

This paper was supported by the KU Research Professor Program of Konkuk University.

*Funding*

This research was funded by Morning Bio Inc. (Republic of Korea).

*Conflicts of interest*

The authors declare they have no conflicts of interest with regard to the work presented in this report.

*Author contributions*

Hyunwoong Jo: Guided the experimental procedures, interpreted data and wrote the manuscript; Jung Yeol Sung: Collected the data, performed statistical analysis and critically revised the manuscript; Beob Gyun Kim: Constructed the research concept and design, and revised and approved the manuscript.

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