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An *In Vitro* Approach to Assess Nutrient Utilization of Brewers Dried Grains and Effects of Supplemental β-Glucanase on Swine Feed Ingredients

Bessem Mariette Akonjuen, Hyunjun Choi and Beob Gyun Kim

Department of Animal Science and Technology, Konkuk University, Seoul, Republic of Korea

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Corresponding Author:
Beob Gyun Kim
Department of Animal Science and Technology,
Konkuk University, Seoul,
Republic of Korea
Email: bgkim@konkuk.ac.kr

Abstract: Two experiments were conducted to determine *In Vitro* Dry Matter Disappearance (IVDMD) of Brewers Dried Grains (BDG) and corn Distillers Dried Grains with Solubles (DDGS) and to investigate effects of supplemental β-glucanase on the IVDMD in barley, BDG and corn DDGS for pigs. In experiment 1, the IVDMD in corn DDGS and two BDG sources produced from Cameroon and Republic of Korea were determined. A three-step IVDMD procedure was employed to simulate digestion of nutrients in the test ingredients by pigs. The digestion 3 steps were for the stomach, the small intestine and the large intestine of pigs. All digestion procedures were conducted in triplicate. The IVDMD in corn DDGS and two BDG sources from Cameroon and Korea was 62.2, 49.2 and 41.0% (±0.6; $p<0.001$), respectively. The IVDMD in corn DDGS was greater ($p<0.05$) than that in BDG sources. The BDG from Cameroon had greater ($p<0.05$) IVDMD than the BDG from Korea. In experiment 2, barley, BDG from Korea and corn DDGS samples were supplemented with 1% β-glucanase (100,000 unit/kg diet) or 1% corn starch. The same IVDMD procedure was used as in experiment 1. While supplemental β-glucanase tended to increase (84.2±0.6 vs. 82.3±0.6; $p = 0.071$) IVDMD in barley, the supplemental enzyme did not affect IVDMD in BDG and corn DDGS. In conclusion, *in vitro* utilization of nutrients in BDG was less efficient than that in corn DDGS and supplemental β-glucanase improves the utilization of nutrients in barley.

Keywords: Barley, Beer Byproducts, Carbohydrase, *In Vitro* Dry Matter Disappearance, Pigs

Introduction

Brewers Dried Grains (BDG) are made from fermentation of barley in beer production (Knudsen, 1997). Other cereal grains could be added to barley for beer production (Mussatto *et al*., 2006). Therefore, the nutrient compositions of BDG vary depending on the inclusion rate of cereal grains for beer production (Robertson *et al*., 2010). Most BDG contain over 25% of crude protein (Davis *et al*., 1983; NRC, 2012). However, the use of BDG in swine diets has been limited (Zhang *et al*., 2013) mainly due to the high fiber concentrations (NRC, 2012).

The use of exogenous enzymes is one of the methods that have shown the potential to improve the nutritional value of high-fiber feed ingredients (Zijlstra *et al*., 2010). The main component for beer production is barley which contains a large quantity of β-glucans. Supplemental β-glucanase has the ability to breakdown intact cell walls and release entrapped nutrients in β-glucans (Lindberg, 2014). However, there is little information on the effects of the β-glucanase on the utilization of BDG and other distillers' by-products.

In vitro assays are widely used to determine the influences of enzyme supplementation on nutrient digestibility because *in vitro* assay data are very highly correlated with data obtained from animal experiments (Park *et al*., 2012). In addition, an *in vitro* assay is a time-saving and inexpensive method compared with *in vivo* experiments (Kong *et al*., 2015; Park *et al*., 2016). Therefore, the objectives of this study were to determine *In Vitro* Dry Matter Disappearance (IVDMD) in two sources of BDG from different countries and corn distillers dried grains with solubles (DDGS) and to investigate effects of supplemental β-glucanase on the IVDMD in barley, BDG and corn DDGS.

Materials and Methods

Test Ingredients and Experimental Designs

The BDG samples were oven-dried at 55°C for five days to be a constant weight. These samples were then finely ground and used for chemical analyses and *in vitro* assays. In experiment 1, the IVDMD in two BDG sources that produced from Cameroon and Republic of Korea and corn DDGS were determined. In experiment 2, effects of β-glucanase on the IVDMD in barley, BDG from Republic of Korea and corn DDGS were determined. The finely ground test ingredients were divided into two groups. Each ingredient group was supplemented with either the enzyme or corn starch at 1%. The concentration of supplemental β-glucanase was 100,000 unit/kg of diet.

In Vitro Disappearance Assays

The *in vitro* digestion assays were based on the *in vitro* digestion technique (Boisen and Fernández, 1997; Park *et al*., 2016). All IVDMD procedures were conducted in triplicate.

Chemical Analyses

The test ingredients were analyzed for crude protein (method 990.03), Dry Matter (DM; method 930.15), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) as described in AOAC (2005).

Calculations and Statistical Analyses

The IVDMD (%) was calculated using the following equation (Park *et al*., 2016):

$$IVDMD(\%) = \left[\left(DM_{TI} - DM_{RS}\right) / DM_{TI}\right] \times 100$$

where, DM_{TI} (g) is the weight of DM in the test ingredient and DM_{RS} (g) is the weight of DM in the undigested residue collected from the IVDMD procedure.

Data were analyzed using the GLM procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA). In experiment 1, the model included the feed ingredient as an independent variable and in experiment 2, the enzyme supplementation was regarded as an independent variable. Differences among least square means were tested using the PDIFF option with Tukey’s adjustment in experiment 1 (Seo *et al*., 2018). Least square means for IVDMD for each ingredient with or without the enzyme supplementation were calculated in experiment 2. The experimental unit was a test flask and the statistical significance of each treatment effect was declared at $p<0.05$ and tendency at $0.05 \leq p<0.10$.

Results

The concentration of crude protein in BDG sources and corn DDGS was greater than that in barley (Table 1). The BDG from Cameroon had greater crude protein and less fiber concentrations than the BDG from Korea.

In experiment 1, the corn DDGS had greater ($p<0.05$) IVDMD compared with BDG from Cameroon and Korea (Table 2). The BDG from Cameroon had greater ($p<0.05$) IVDMD than the BDG from Korea.

Table 1: Analyzed nutrient composition of barley, two sources of brewers dried grains and corn distillers dried grain with solubles (DDGS)[1], as-is basis

		Brewers dried grains		
Item	Barley	Cameroon	Korea	Corn DDGS
Dry matter, %	90.7	97.9	93.9	87.4
Crude protein, %	7.0	31.9	26.4	26.8
NDF[2], %	21.3	54.0	58.2	35.4
ADF[3], %	10.5	22.2	25.2	7.3

[1]Data are the mean of triplicate analyses of each ingredient
[2]NDF = neutral detergent fiber
[3]ADF = acid detergent fiber

Table 2: *In vitro* dry matter disappearance (IVDMD, %) of brewers dried grains (BDG) from Cameroon and Korea and corn distillers dried grain with solubles (DDGS), experiment 1[1]

	BDG				
Item	Cameroon	Korea	Corn DDGS	SEM[2]	p-value
IVDMD	49.2[b]	41.0[c]	62.2[a]	0.6	<0.001

[a-c]Means within a row without a common superscript letter differ ($p<0.05$)
[1]Each least square means represents 3 observations
[2]SEM = standard error of the means

Table 3: *In vitro* dry matter disappearance (%) of barley, brewers dried grains (BDG) and corn distillers dried grains with solubles (DDGS) with or without β-glucanase supplementation, experiment 2[1]

Item	Control	β-glucanase	SEM[2]	p-value
Barley	82.3	84.2	0.6	0.071
BDG, Korea	42.2	42.5	0.6	0.683
Corn DDGS	63.0	63.7	0.4	0.309

[1]Each least square means represent 3 observations
[2]SEM = standard error of the means

In experiment 2, supplemental β-glucanase tended to increase ($p = 0.071$) the IVDMD in barley (Table 3). However, the supplementation of β-glucanase did not affect the IVDMD in BDG or corn DDGS.

Discussion

Chemical Composition of Ingredients

The crude protein, NDF and ADF concentrations in BDG from Korea agree with the values in previous studies (Murdock *et al*., 1981; Aghajanzadeh-Golshani *et al*., 2010; Soriano *et al*., 2014). However, BDG from Cameroon had a relatively high crude protein concentration compared with values in the literature (Murdock *et al*., 1981; Aghajanzadeh-Golshani *et al*., 2010; Soriano *et al*., 2014). The deviations of nutrient composition among BDG sources are likely due to the different inclusion rates of cereal grains for beer production and the different beer production procedures. Brewers dried grains are mainly made from the fermentation of barley for beer production, but other cereal grains such as corn, rice or wheat are often added (Mussatto *et al*., 2006). In addition, the nutrient composition of BDG varies depending on harvest time, malting, mashing conditions and processing techniques (Robertson *et al*., 2010). In the present work, however, we were unable to obtain concrete information on the beer procedures and usages of cereal grains.

In Vitro Dry Matter Disappearance of Feed Ingredients (Experiment 1)

Dietary fiber negatively affects nutrient digestibility in pigs (Park *et al*., 2012; Son *et al*., 2012; 2017). The lower IVDMD value for two BDG sources compared with corn DDGS is likely due to their high fiber concentrations (Son *et al*., 2017). The different IVDMD of the two BDG sources can also be explained by different fiber concentrations (Table 1).

Effects of β-glucanase on In Vitro Dry Matter Disappearance (Experiment 2)

The increased IVDMD in barley by supplemental β-glucanase was in line with the previous studies, which demonstrated an increase in the digestibility of nutrients when barley-based swine diets were supplemented with β-glucanase (Inborr *et al*., 1993; Yin *et al*., 2001). The endosperm cell wall of barley consists mainly of 70 to 75% of β-glucans (Åaman and Graham, 1987; Knudsen, 1997) and these β-glucans physically limit access of digestive enzymes to the inside of the cell (Pettersson and Åman, 1988; Grundy *et al*., 2016). Beta-glucanase has been reported to breakdown β-glucans in the endosperm cell wall of barley and to liberate the nutrients therein (Li *et al*., 1996), which may increase nutrients digestibility.

In present study, we failed to find the effects of supplementation of β-glucanase on the IVDMD in BDG or corn DDGS. The reason for this might be that BDG contains less amounts of β-glucans (less than 1%) than those of barley (Ikram *et al*., 2017), thus there may be little substrate for the enzyme to work on. Exogenous enzymes do not improve nutrient utilization in diets containing insufficient amount of substrates (Zijlstra *et al*., 2010; Kwon and Kim, 2015; Park *et al*., 2016). Also, BDG had high concentrations of hemicellulose and lignocellulose (Ikram *et al*., 2017). The lignocellulose matrix has a rigid structure that may inhibit the action of enzyme (Lynch *et al*., 2016). Therefore, these factors might have prevented β-glucanase from being active. No effects of supplemental β-glucanase can also be explained by the limited substrate quantity. Most corn by-products contain relatively high arabinoxylan concentrations, but less β-glucans concentrations (Knudsen, 1997). The present results agree with Jones *et al*. (2010) who reported that the supplementation of an enzyme complex (β-glucanase, galactomannanase and xylanase) to diets containing DDGS did not improve the growth performance in pigs. Therefore, an inadequate quantity of substrates for β-glucanase limits the effects of β-glucanase on nutrient utilization in BDG and corn DDGS.

Conclusion

In vitro utilization of nutrients in brewers dried grains was less efficient than that of corn distillers dried grains with solubles and β-glucanase improved *in vitro* dry matter disappearance of barley. Further research is warranted to investigate nutrient utilization of brewers dried grains and effects of supplemental β-glucanase.

Author's Contributions

Bessem Mariette Akonjuen: Conducted the experiment and prepared the manuscript draft.

Hyunjun Choi: Performed statistical analysis and critically revised the manuscript.

Beob Gyun Kim: Supervised the experimental work and manuscript preparation and revised the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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