J. Dairy Sci. 99:3602–3606
http://dx.doi.org/10.3168/jds.2015-9904

Short communication: The effect of an exogenous enzyme with amylolytic activity on gas production and in vitro rumen starch degradability of small and large particles of corn or barley meals

A. Gallo,* G. Giuberti,* S. Duval,† M. Moschini,* and F. Masoero*[1]
*Feed & Food Science and Nutrition Institute, Faculty of Agriculture, Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy
†DSM Nutritional Products, 1 Bd d'Alsace, Village-Neuf. BP170, France

ABSTRACT

The objective of this study was to evaluate the effect of exogenous amylase supplementation on gas production and on in vitro rumen starch degradability (IVSD) of different sized particles of corn and barley meals (Cm and Bm, respectively). An aqueous liquid amylase formulation from *Bacillus licheniformis* was tested at 3 enzyme doses (EnzD; 0, 300 and 1,500 kilo novo units/kg of dry matter) on small (<750 μm) and large (≥750 μm) particle size (PS) of Cm and Bm. Data were analyzed according to a randomized complete block design with a factorial arrangement of treatments; the main tested effects were PS, EnzD, and their interaction. Fermentation run entered in the model as random effect. The mixed rumen fluid was collected from 2 rumen-fistulated Holstein dry dairy cows fed at maintenance (forage:concentrate ratio of 80:20; 12% crude protein; 55% amylase-treated neutral detergent fiber). Small particles of both Cm and Bm had a greater rate of fermentation and shorter lag time than large particles. The rate of starch degradation was greater for small than for large particles of Bm, being 0.187 and 0.125 1/h, respectively. Conversely, the rate of starch degradation of Cm averaged 0.063 1/h and was similar among treatments. Enzyme supplementation tended to reduce lag time and to increase rate of fermentation for both PS of Cm and Bm, with a more pronounced effect for small PS. A limited EnzD effect was measured for IVSD data and rate of starch degradation; PS influenced fermentation parameters and the magnitude of starch degradation more than EnzD. Supplementation with exogenous amylase influenced the rumen fermentation pattern of small and large PS of Cm and Bm, even if the effect of the enzyme supplementation differed according to the PS of cereal meals.

Key words: exogenous amylase, particle size, gas production, starch degradation

Short Communication

Cereals represent the primary starch source in ruminant diets and relatively high levels of dietary starch are typically used to meet the energy requirements of high-producing lactating dairy cows (Giuberti et al., 2014). A greater understanding of starch digestion, along with variation in raw ingredient composition and the historic increase in cereal prices (FAO, 2015), has increased interest in using exogenous amylase in dairy cow diets (Tricarico et al., 2007), even if possible benefits in increasing rumen starch digestibility should be evaluated against potential digestive disorders in cattle (Yang and Beauchemin, 2006). Variable responses following enzyme supplementation have been reported. In particular, Gencoglu et al. (2010) reported a greater conversion of feed to milk in dairy cows fed reduced-starch diets (dietary starch content from 27.1 to 20.7% DM) with inclusion of a commercial exogenous amylase. Ferraretto et al. (2011) and Weiss et al. (2011), carrying out in vivo experiments, described marginal beneficial effects due to the addition of the same commercial enzyme in normal or reduced-starch diets (dietary starch content of 27 to 31% or 21 to 26% DM, respectively). As hypothesized by Weiss et al. (2011), a potential reason for the lack of effect of amylolytic enzyme supplementation could be related to the effect of particle size (**PS**) of the grains, hiding possible effects resulting from the enzyme supplementation.

Because no information is currently available on the effect of exogenous amylase supplementation in relation to different PS of cereal grains, the current study was designed to evaluate the effects of enzyme supplementation on gas production and in vitro rumen starch degradability (**IVSD**) of small (<750 μm) and large (≥750 μm) particles of corn and barley meals (**Cm** and **Bm**, respectively). These in vitro rumen-based methods are useful to evaluate potential digestibility of nutrients

Received June 3, 2015.
Accepted October 1, 2015.
[1]Corresponding author: francesco.masoero@unicatt.it

in the different compartments of the gastrointestinal tract of animals (Giuberti et al., 2014).

Three 5-kg batches of dry whole-kernel corn (Cm) and dry, hull-less whole-kernel barley (Bm) were collected over 3 wk (one batch each week) from the same feedstock grains stored in 2 silos on the CERZOO Research and Experimental Dairy Farm (San Bonico, Italy). Subsamples (1 kg) of Cm and Bm were separately processed through a 2-mm sieve using a Retsch type ZM100 centrifugal grinding mill (Retsch, Haan, Germany). Representative aliquots (200 g) of the resulting products were run through a sieve shaker (RX-29-E-110, W. S. Tyler Co., Mentor, OH) equipped with a 750-μm sieve for 10 min. Particles were thus divided into small (<750 μm) and large (≥750 μm) PS. Another portion (500 g) of each of the 3 batches of cereal grain was ground through a 1-mm screen and stored for further analysis, as previously described by Gallo et al. (2013). Samples were assayed according to AOAC International (2000) methods for DM, CP, ether extract, and ash contents (methods 930.15, 976.05, 954.02 without acid hydrolysis, and 942.05, respectively). Total starch content was determined by polarimetry (Polax 2L, Atago, Tokyo, Japan). Amylase-treated NDF (**aNDF**), analyzed by using heat-stable amylase and sodium sulfite and not corrected for residual ash, and the ADF contents of samples were determined as described by Van Soest et al. (1991). All analyses were assayed in duplicate for each cereal batch (except for DM determination) and values were averaged as analytical replicates.

An aqueous liquid amylase formulation from *Bacillus licheniformis* (DSM 21564; Ronozyme RumiStar 240L, DSM Nutritional Products, Basel, Switzerland; Novozyme A/S, Bagsvaerd, Denmark) with a minimum declared amylase activity of 240 kilo novo units (**KNU**)/g (EFSA, 2012) was tested at 3 enzyme doses (**EnzD**): 0, 300, and 1,500 KNU/kg of DM, corresponding, respectively, to approximately 0, 0.28, and 1.38 mg per syringe or 0, 0.43, and 2.16 mg per bottle for Cm, and 0, 0.50, and 2.50 mg per bottle for Bm of the commercial product) on the 2 PS (<750 and ≥750 μm) produced from each batch of Cm and Bm. Specific amounts of the commercial product were previously weighed into 3-mL graduated plastic syringes and then individually injected to each corresponding syringe or bottle before addition of diluted rumen fluid.

Samples were incubated with diluted rumen fluid to measure rumen fermentation according to gas production as described by Menke and Steingass (1988). Rumen liquor was collected from the rumen of 2 fistulated Holstein dry dairy cows (625 ± 10 kg of BW, 38 ± 0.1 mo old) fed at maintenance (NRC, 2001) with a TMR (12% CP and 55% aNDF on a DM basis) composed of grass hay, corn silage, and a protein vitamin mineral supplement (75, 15, and 10% DM, respectively). The collected rumen liquor was filtered through 2 layers of cheesecloth before being diluted with buffer (buffer:rumen fluid ratio of 2:1, vol:vol) and saturated with CO_2, and then the pH was corrected to 6.5 to 6.6. At all times, the rumen liquor was maintained in a warm insulated flask and used within 20 min of sampling. In the meantime, 220 mg of each sample to be tested was weighed into a graduated 100-mL glass syringe and 30 mL of the diluted rumen fluid was added. Syringes were placed vertically in a water bath at 39°C and 3 syringes without substrate were used as blanks. Samples were incubated in triplicate in 2 separate runs. Gas production was measured after 1, 2, 4, 6, 8, 12, 24, 48, and 72 h of incubation. An internal standard was also incubated. A one-pool exponential model was adopted (Wang et al., 2013) to fit gas production data and obtain curve parameters—final volume (**Vf**, mL/g of OM), rate of gas production (**kd**, 1/h), and lag time (h)—for each individual syringe.

The IVSD was evaluated by an in vitro method proposed by Sveinbjörnsson et al. (2007) and modified by Gallo et al. (2014). To incubate a similar amount of starch (i.e., 250 mg), each sample was weighed into 125-mL glass bottles equipped with rubber stoppers. Then, the samples were gassed with CO_2 and incubated with 30 mL of the buffered rumen fluid (described above) at 39°C in a shaking water bath at 50 rpm. Blanks and internal standard (Gelose 80 maize starch; Penford Food Ingredients Co., Centennial, CO) were also incubated. The IVSD was calculated as the ratio between the amount of starch that disappeared after 2, 4, 7, and 72 h of incubation and the amount of starch in the sample before incubation, after correction for blanks (Sveinbjörnsson et al., 2007). Samples were analyzed in triplicate in 2 separate runs. Three bottles were used for each time point. At each experimental time, bottles were plunged into a bath of ice to stop fermentation, and the remaining starch was quantified by a 2-step enzymatic (i.e., α-amylase and amyloglucosidase hydrolysis) approach (Gallo et al., 2014).

The rate of starch degradation (**kd starch**, 1/h) was calculated from IVSD data measured after 2, 4, and 7 h of rumen incubation, using a first-order exponential model and assuming a constant lag time of 0.5 h and a constant amount of indigestible starch equal to 0.5% starch (IVSD values evaluated after 72 h of rumen incubation were lower than 1% for all samples).

Using the MIXED procedure of SAS (SAS Institute, 2003), ANOVA was conducted using a completely randomized block design for each tested meal (i.e., Cm or Bm) with a factorial arrangement of treatments.