J. Dairy Sci. 101:1–8
https://doi.org/10.3168/jds.2017-13944

Short communication: In vitro rumen gas production and starch degradation of starch-based feeds depend on mean particle size

A. Gallo,*[1] G. Giuberti,* A. S. Atzori,† and F. Masoero*
*Department of Animal Science, Food and Nutrition (DIANA), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy
†Dipartimento di Agraria, Sezione di Scienze Zootecniche, Università di Sassari, 07100 Sassari, Italy

ABSTRACT

Our objective was to model the effect of mean particle size (mPS) on in vitro rumen starch degradation (IVSD) and the kinetics of gas production for different starch-based feeds. For each feed, 2 batches of the same grains were separately processed through 2 different mills (cutter or rotor speed mills), with or without different screens to achieve a wide range of mPS (0.32 to 3.31 mm for corn meals; 0.19 to 2.81 mm for barley meals; 0.16 to 2.13 mm for wheat meals; 0.28 to 2.32 mm for oat meals; 0.21 to 2.36 mm for rye meals; 0.40 to 1.79 for sorghum meals; 0.26 to 4.71 mm for pea meals; and 0.25 to 4.53 mm for faba meals). The IVSD data and gas production kinetics, obtained by fitting to a single-pool exponential model, were analyzed using a completely randomized design, in which the main tested effect was mPS (n = 6 for all tested meals, except n = 7 for corn meals and n = 5 for sorghum meals). Rumen inocula were collected from 2 fistulated Holstein dairy cows that were fed a total mixed ration consisting of 16.2% crude protein, 28.5% starch, and 35.0% neutral detergent fiber on a dry matter basis. The IVSD, evaluated after 7 h of rumen incubation, decreased linearly with increasing mPS for corn, barley, wheat, rye, pea, and faba meals, and decreased quadratically with increasing mPS for the other meals. The y-axis intercept for 7-h IVSD was below 90% starch for corn, barley, and rye feeds and greater than 90% for the other tested feeds. The mPS adjustment factors for the rate of rumen starch degradation varied widely among the different tested feeds. We found a linear decrease in starch degradation with increasing mPS for barley, wheat, rye, and pea meals, whereas we noted a quadratic decrease in starch degradation for the other tested meals. Further, we observed a linear decrease in the rate of gas production with increasing mPS in each tested feed, except for pea meal, which had a quadratic relationship. For each 1 mm increase in mPS, the gas production was adjusted by -0.009 h^{-1} for corn, -0.011 h^{-1} for barley, -0.008 h^{-1} for wheat, and -0.006 h^{-1} for faba, whereas numerically greater adjustments were needed for oat (-0.022 h^{-1}), rye (-0.017 h^{-1}), and sorghum (-0.014 h^{-1}). These mPS adjustment factors could be used to modify the starch-based feed energy values as a function of mean particle size, although in vivo validation is required.

Key words: in vitro method, processing, fermentation kinetics, nutritional model

Short Communication

Farmers typically give high-energy diets to lactating dairy cows. However, diets that are rapidly fermented in the rumen can lead to the rapid production of VFA. If the production of these acids exceeds the ability of the rumen to neutralize and absorb them, SARA can occur, thus worsening microbial fermentation, rumen epithelial function, animal health, and milk production (Silveira et al., 2007; Penner et al., 2009). Therefore, many developers of ruminant nutrition models have recently focused on prediction of starch digestion dynamics in the digestive tracts of dairy cows (Higgs et al., 2015; Bannink et al., 2016; Ghimire et al., 2017). Two recent meta-analyses (Patton et al., 2012; Moharrery et al., 2014) aimed to determine the amount of starch digested in the different compartments of the gastrointestinal tract and to identify the main factors affecting starch digestion dynamics. In both cases, the proposed starch digestion submodels were mainly based on starch sources and starch intake levels. Nonetheless, it was difficult to include certain other factors in these models. Thus, Patton et al. (2012) declared "...inaccuracies in prediction of starch degradability in the rumen may be mainly due to processing effects and particle sizes, but these were not well reported in literature and were difficult to estimate," and Moharrery et al. (2014) stated "...effects of physical structure and heat treatment were initially tested, however data balance

Received October 5, 2017.
Accepted March 14, 2018.
[1]Corresponding author: antonio.gallo@unicatt.it

did not allow for conclusive statements for the present dataset and approach."

Previous studies suggested that the mean particle size (**mPS**) of feeds can affect digestion rate (Hoffman et al., 2012; Zhao et al., 2015; Tagawa et al., 2017), and that differences in the passage rates of large, medium, and small particles exist within the different gastrointestinal compartments of ruminant animals (Nocek and Tamminga, 1991; Offner and Sauvant, 2004; Ferraretto et al., 2013). We previously modeled the effects of mPS on in vitro rumen starch degradation (**IVSD**) and OM fermentability (Gallo et al., 2016) by examination of a wide range of mPS, with meals consisting of dry whole-kernel corn (n = 11 mPS, 0.46 to 3.50 mm) and dry hulless whole-kernel barley (n = 10 mPS, 0.11 to 2.98 mm), by use of well-established rumen-based in vitro methods. We proposed the use of mPS adjustment factors (i.e., the slopes of regression terms in which mPS represented the independent variable) to characterize the rate of rumen starch degradation (**kd starch**) and the amount of starch degradation after 7 h of in vitro rumen incubation (**7hIVSD**). In particular, the previous study indicated that for each 1-mm increase in mPS, corn feed had a linear decrease of 0.049 h^{-1} in the kd starch and a 6.3 percentage units decrease in the 7hIVSD, and barley feed had a linear decrease of 0.092 h^{-1} in the kd starch and a 6.5 percentage units decrease in the 7hIVSD.

The purpose of the current study was to extend the previous approach to other starch-based feeds, with the aim to model the effect of mPS on IVSD data and the kinetics of gas production. In particular, we selected the same raw starch-based feeds examined in the previous meta-analysis of Moharrery et al. (2014).

Two 5-kg batches of dry whole corn, dry hulled barley, dry wheat, dry oat, dry rye, and dry sorghum kernels were collected over 2 wk (1 batch each week) from the same feedstock grains stored in grain storage bins at local industrial feed mills. Two batches of dry whole pea (*Pisum sativum*) and faba (*Vicia faba* var. minor) beans were generously donated by the Centro Ricerche Produzioni Animali S.p.A. (CRPA, Reggio Emilia, Italy).

To obtain different mPS, subsamples of about 1.3 to 1.5 kg for each batch were processed as described by Hoffman et al. (2012), in which a cutter mill (Pulviresette 19, Fritsch, Idar-Oberstein, Germany) that was fitted with 4.0-, 3.0-, 2.0-, or 1.0-mm screens (1 passage) or without screens (1 to 5 passages) was used. Samples were also passed through a rotor speed mill Pulverisette 14 (Fritsch) that was equipped with 1.0- and 0.5-mm screens (1 passage; Table 1). Afterward, a representative amount (100 g) of the various grinds was run for 10 min through a sieve shaker (Multidimensional Sieveshaker IG/1/S, Giuliani Tecnologie s.r.l., Torino, Italy) that had 9 different screen sieves with nominal aperture sizes of 4.00, 3.50, 2.50, 1.50, 1.00, 0.75, 0.50, 0.25, and 0.125 mm, followed by a pan. The mPS was measured using equation 1 of ASAE S319.3 method, as reported in ASABE (2006). In particular, the mPS of each material retained on a sieve was calculated on a weight basis as the geometric mean of the diameter of the openings in the 2 adjacent sieves in the stack (Pfost and Headley, 1976). A portion of each subsample that was ground through a screen of over 0.50 mm was reground by the rotor speed mill equipped with 0.50-mm screen and analyzed for total starch (Megazyme assay kit K-TSTA 07/11), ash (AOAC International, 2000; method 942.05), and CP (AOAC International, 2000; method 984.13).

The IVSD was evaluated by an in vitro rumen-based method, which was slightly modified from the method of Sveinbjörnsson et al. (2007). Rumen fluid was collected from 2 fistulated dairy cows that received a TMR (16.2% CP, 28.5% starch, and 35.0% NDF on a DM basis), formulated according to the NRC (2001) for an average BW of 600 kg, 140 DIM, and 35 kg of milk yield (3.75% fat and 3.35% protein). The diet consisted of corn silage, energy-protein supplement, alfalfa, and grass hays (31.2, 48.0, 16.7, and 4.1% DM, respectively). Rumen liquor was maintained in a warm insulated flask, filtered through 2 layers of cheesecloth, and used within 20 min of collection. Samples containing 250 mg of starch were weighed in 125-mL glass bottles (Wheaton borosilicate glass serum bottle; 54 mm diameter × 107 mm height; Z114014; Sigma-Aldrich Co., Milan, Italy), which were filled with 30 mL of the diluted rumen fluid (buffer-to-rumen ratio 2:1, vol/vol), gassed with CO_2, closed with rubber stoppers (gray butyl stoppers; 20-mm diameter; 27232; Sigma-Aldrich Co.), and then incubated at 39°C in a shaking water bath (50 rpm). Blank samples (diluted rumen fluid only) and an internal standard (Gelose 80 maize starch; Penford Food Ingredients Co., Englewood, CO) were also included. After different incubation times, bottles were plunged into a bath containing ice to stop starch degradation. Residual starch was quantified using a 2-step enzymatic approach detailed previously (Gallo et al., 2016).

The IVSD after 7 or 120 h of rumen incubation was calculated as

$$\text{IVSD, \% starch} = [1 - (\text{resStarch} - \text{blnStarch})/\text{incStarch}] \times 100\%, \quad [1]$$

where resStarch is the amount of residual starch after 7 or 120 h of rumen incubation; blnStarch is the blank