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## Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products

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### ABSTRACT

The objectives of this study were to evaluate the dynamics of gas production of several corn (co)products, to develop equations to predict the rate of ruminal fiber digestion, to estimate total digestible nutrients (TDN) and net energy for lactation (NE<sub>L</sub>), and to assess the stochasticity of chemical composition and nutritive value variability. Four corn milling (co)products were evaluated in this study: high protein dried distillers grains (HP-DDG), corn bran (BRAN) and dehydrated germ (GERM), and a dried distillers grains plus soluble produced with a low-heat drying process (BPX). Alfalfa hay was used as an internal standard feed in the *in vitro* fermentation dynamics analysis. Standard chemical analyses, *in vitro* digestibility, and *in vitro* gas production techniques were used to obtain the necessary physicochemical characterization of feeds. The *in vitro* dry matter digestibility at 24 and 48 h of incubation decreased exponentially as acid detergent insoluble nitrogen increased. However, the degree of *in vitro* dry matter digestibility reduction was more accentuated at 24 than at 48 h of incubation. The difference among these feeds regarding the dynamics of the anaerobic fermentation within different substrates (intact feed, and fiber and defatted residues) was evaluated. Results suggested that the proportion of fiber digested in the rumen was affected by the degree of sample processing and fat removal. Fractional fermentation rate (kf) of neutral detergent residue (without sodium sulfite) and defatted fiber residue for BRAN, GERM, HP-DDG, and BPX was estimated to be 0.0635 and 0.0852 h<sup>-1</sup>, 0.0803 and 0.0914 h<sup>-1</sup>, 0.118 and 0.117 h<sup>-1</sup>, and 0.0695 and 0.0844 h<sup>-1</sup>, respectively. The most influential variables affecting kf<sub>NDR</sub> of HP-DDG and BPX also affected the

predicted TDN, suggesting that fiber quality is essential to ensure higher TDN values for these feeds. Our study indicated that it is possible to routinely quantify the rate of fiber digestion and this approach may be based on common analytical procedures namely estimates of neutral detergent fiber, acid detergent fiber, acid detergent insoluble nitrogen, ether extract, and acid detergent lignin. Our simulations of TDN values demonstrated that differences in fermentability and chemical composition of these corn (co)products might considerably affect the supply of energy to lactating dairy cow. The analytical methods developed in this study may serve as a valuable tool to assess nutrient quality and uniformity when samples differ in chemical composition.

**Key words:** digestibility, feed composition, fermentation, Monte Carlo simulation

### INTRODUCTION

Different (co)products are obtained from the fuel-ethanol dry milling industry depending upon the process used during the fermentation of corn to ethanol. These (co)products have been used in ruminant diets. Schingoethe et al. (1999) fed wet corn distillers grains to Holstein cows and found no effect on milk production, but DMI was lower, milk fat was slightly greater, and milk protein was lower. Similarly, dried distillers grains plus soluble (DDGS) has been shown to decrease DMI and increase milk yield while maintaining milk fat percentage (Anderson et al., 2006), and increase feed efficiency (Kleinschmit et al., 2006) compared with control diets. Corn (co)products can be effective substitutes for corn grain and soybean meal (Sasikala-Appukuttan et al., 2008) for lactating dairy cows provided that limiting amino acids (e.g., Lys, Met, and Phe) are supplemented (Nichols et al., 1998). For nursing beef calves, DDGS can be partially equivalent to soybean meal, soybean hulls, and wheat middlings (Reed et al., 2006).

Corn (co)products can be used in diet formulation to ensure that dietary energy and nutrients closely match the requirements of producing animals. Linear (Tede-

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schi et al., 2000) and stochastic (Tozer, 2000) programming have been used to formulate diets. However, the distribution of nutrients in the ingredients is needed in stochastic programming (Tozer, 2000) and the variation of feed chemical composition may be used to establish optimal sampling schedules of ingredients for chemical analysis to keep milk production within targeted levels and to achieve maximal economical returns (St-Pierre and Cobanov, 2007).

Even though corn (co)products have been routinely used in dairy rations, the inclusion levels of these products are conservatively low (less than 10% of the diet). Despite being a good source of digestible fiber, protein, and energy, the inclusion of these feedstuffs is limited because they may vary considerably in their nutrient content and availability. The objectives of this experiment were to evaluate the variability in chemical composition, to determine the in vitro fermentation kinetics, and to estimate total digestible nutrients (TDN) variability based on the chemical composition of 4 corn milling (co)products.

## MATERIALS AND METHODS

### *Description of the Products*

Corn ethanol production is made up of multistep processes. Initially, corn is ground and water is added to produce a mash. In the second step, the mash is heated and cooled, followed by a yeast-based fermentation. After the fermentation, the ethanol-rich mash is transferred to distillation columns that separate the ethanol from the stillage. The stillage is centrifuged to separate the coarse grain and solubles. Last, through evaporation, solubles are concentrated to about 30% of DM and then added to the coarse grain to be dried together thereby producing DDGS.

A unique process that aims to improve ethanol yield and nutritional value of resulting (co)products has been developed by Poet LLC (Sioux Falls, SD). More specifically, the use of heat before fermentation has been avoided, resulting in a (co)product (Dakota Gold BPX; **BPX**) that is less likely to contain heat-damaged proteins. Heat-damaged proteins have lower digestibility in ruminants (Krishnamoorthy et al., 1982). In addition, another process that promotes the physical removal of bran (Dakota Bran; **BRAN**) and germ (Dakota Gold Corn Germ Dehydrated; **GERM**) before fermentation has been developed. This process also results in a fourth corn milling (co)product (Dakota Gold HP DDG; **HP-DDG**) that is similar to the traditional process, but the bran and germ have been removed and the starch has been fermented. Solubles resulting from these processes are added to the BRAN and GERM (co)products.

Four corn milling (co)products were evaluated in this study: HP-DDG (TCE LLC, Coon Rapids, IA), BRAN (EXOL, Albert Lea, MN), GERM (TCE LLC), and BPX (Northstar Ethanol LLC, Lake Crystal, MN) (Table 1). At each plant, 2 samples were collected each week (from January 2007 to April 2007) until a total of 30 samples for each corn milling (co)product were collected. Feed identification numbers were assigned sequentially to corn milling (co)products as they were sampled. Additionally, alfalfa hay (**HAY**) was used as a standard feed in the in vitro gas production dynamics analysis for comparative purposes among fermentation runs (laboratory control). When the dynamics of gas production of the standard alfalfa deviated from the average gas production pattern, the run was discarded and repeated. The difference among these feeds regarding the dynamics of the anaerobic fermentation within different substrates (intact feed, fiber residues, and defatted residues) was evaluated. The neutral detergent residue (**NDR**; Hintz et al., 1996; Mertens, 2003) provides information about the quality of the fiber in supporting microbial growth whereas the defatted residue (**DFR**) provides information regarding the impact of ether extract (**EE**) of the feed in the dynamics of fermentation. It is well known that fat can greatly influence fermentation as evidenced by reduced digestibility of fibrous matter, decreased microbial growth, and alteration of fermentation products (Nagaraja et al., 1997). Even though feeding corn milling (co) products may not have these detrimental effects in producing animals, there might be a negative impact on in vitro anaerobic fermentation conditions (Chalupa et al., 1984).

### *Chemical and In Vitro Methods*

**Fiber Residue.** Upon arrival at the University of Nebraska Ruminant Nutrition Laboratory, approximately 300 g of each sample was ground by hand using a marble mortar and pestle. For in vitro anaerobic fermentations (in vitro digestibility and in vitro gas production procedures), fiber was obtained by using neutral detergent solution (FND20C; Ankom Technology, Macedon, NY) with heat-stable  $\alpha$ -amylase (100  $\mu$ L per 0.50 g of sample; Ankom Technology) but without sodium sulfite ( $\text{Na}_2\text{SO}_3$ ). Therefore, this fiber residue is referred to as NDR to differentiate it from the AOAC (2000; method 2002.04) for NDF and  $\alpha$ -amylase-treated NDF (**aNDF**; Mertens, 2002), which uses sodium sulfite.

**Defatted Residue.** Defatted residue was obtained (AOAC, 2000; method 971.09) and used in the in vitro anaerobic fermentation and gas production procedure. Extraction was performed using a 1,000-mL Soxhlet extractor and Friedrichs condenser. Original samples (2

**Table 1.** Chemical characterization of four corn milling (co)products<sup>1</sup>

| Items <sup>2</sup>                                  | Mean ± SD         |                   |                   |                   | 90% Confidence interval |                    |                    |                    |
|---|-------------------|-------------------|-------------------|-------------------|-------------------------|--------------------|--------------------|--------------------|
|   | BRAN              | GERM              | HP-DDG            | BPX               | BRAN                    | GERM               | HP-DDG             | BPX                |
| DM, % as-fed  | 31<br>90.3 ± 1.15 | 30<br>94.8 ± 1.05 | 29<br>90.8 ± 1.78 | 30<br>91.4 ± 0.94 | 31<br>[89.9, 90.7]      | 30<br>[94.4, 95.2] | 29<br>[90.1, 91.5] | 30<br>[91.0, 91.7] |
| CP, % of DM   | 15.3 ± 0.67       | 17.4 ± 0.79       | 44.6 ± 1.43       | 30.8 ± 0.57       | [15.1, 15.6]            | [17.1, 17.7]       | [44.1, 45.2]       | [30.6, 31.0]       |
| Ether extract, % of DM                              | 9.49 ± 0.82       | 17.4 ± 1.70       | 4.18 ± 0.43       | 11.2 ± 0.47       | [9.19, 9.79]            | [16.7, 18.0]       | [4.02, 4.34]       | [11.0, 11.4]       |
| Ash, % of DM  | 3.84 ± 0.42       | 6.01 ± 0.45       | 1.90 ± 0.20       | 4.19 ± 0.33       | [3.68, 3.99]            | [5.84, 6.18]       | [1.82, 1.98]       | [4.06, 4.31]       |
| Neutral detergent fiber (aNDF), % of DM             | 21.4 ± 1.75       | 30.1 ± 3.81       | 27.3 ± 3.46       | 30.0 ± 3.25       | [21.1, 22.7]            | [32.0, 35.1]       | [33.4, 36.3]       | [32.3, 33.5]       |
| Neutral detergent residue (NDR), % of DM            | 21.9 ± 2.21       | 33.5 ± 4.15       | 34.8 ± 3.80       | 32.9 ± 1.62       | [21.1, 22.7]            | [32.0, 35.1]       | [33.4, 36.3]       | [32.3, 33.5]       |
| ADF, % of DM  | 7.36 ± 1.53       | 15.1 ± 2.32       | 20.4 ± 5.29       | 12.8 ± 1.60       | [6.80, 7.92]            | [14.2, 16.0]       | [18.4, 22.4]       | [12.2, 13.4]       |
| ADIN, % of CP                                       | 1.99 ± 1.03       | 2.07 ± 0.71       | 5.82 ± 2.18       | 2.97 ± 1.05       | [1.61, 2.37]            | [1.81, 2.34]       | [4.98, 6.65]       | [2.58, 3.37]       |
| ADL, % of NDR                                       | 12.0 ± 4.67       | 9.3 ± 3.02        | 11.5 ± 3.47       | 12.3 ± 3.41       | [10.3, 13.8]            | [8.18, 10.4]       | [10.2, 12.8]       | [11.0, 13.6]       |
| In vitro digestibility, %                           |                   |                   |                   |                   |                         |                    |                    |                    |
| DM at 24 h  | 67.3 ± 3.60       | 69.6 ± 5.36       | 42.2 ± 3.85       | 54.2 ± 3.06       | [65.7, 68.9]            | [67.5, 71.6]       | [40.6, 43.8]       | [53.0, 55.4]       |
| DM at 48 h  | 77.0 ± 2.29       | 78.1 ± 4.43       | 62.5 ± 3.80       | 68.8 ± 4.25       | [76.0, 77.9]            | [76.4, 79.9]       | [60.9, 64.1]       | [67.1, 70.4]       |
| NDR at 24 h   | 79.8 ± 2.09       | 83.1 ± 3.01       | 70.6 ± 5.81       | 75.6 ± 3.59       | [79.1, 80.6]            | [82.0, 84.2]       | [68.3, 72.8]       | [74.2, 76.9]       |
| NDR at 48 h   | 86.6 ± 1.78       | 89.6 ± 3.10       | 84.8 ± 5.11       | 86.3 ± 2.73       | [86.0, 87.3]            | [88.5, 90.8]       | [82.8, 86.7]       | [85.3, 87.4]       |
| Fractional rate of disappearance <sup>3</sup> , %/h |                   |                   |                   |                   |                         |                    |                    |                    |
| Estimated from DM at 24 h                           | 4.69 ± 0.457      | 5.03 ± 0.795      | 2.29 ± 0.285      | 3.26 ± 0.280      | [4.48, 4.89]            | [4.72, 5.33]       | [2.17, 2.41]       | [3.15, 3.37]       |
| Estimated from DM at 48 h                           | 3.07 ± 0.211      | 3.21 ± 0.442      | 2.05 ± 0.213      | 2.44 ± 0.295      | [2.98, 3.16]            | [3.03, 3.38]       | [1.96, 2.14]       | [2.33, 2.56]       |
| Estimated from NDR at 24 h                          | 6.70 ± 0.436      | 7.47 ± 0.761      | 5.19 ± 0.991      | 5.92 ± 0.660      | [6.53, 6.86]            | [7.18, 7.75]       | [4.81, 5.58]       | [5.68, 6.17]       |
| Estimated from NDR at 48 h                          | 4.21 ± 0.276      | 4.81 ± 0.619      | 4.03 ± 0.728      | 4.19 ± 0.411      | [4.11, 4.31]            | [4.58, 5.04]       | [3.76, 4.31]       | [4.03, 4.34]       |

<sup>1</sup>BRAN = bran, GERM = corn germ dehydrated. HP-DDG and BPX are corn dried distillers grain (co)products in which BPX undergoes a low heat process and HP-DDG has high protein content.

<sup>2</sup>NDR is without sodium sulfite and NDF is with sodium sulfite; both contain α-amylase.

<sup>3</sup>Computed using exponential nonlinear equation without lag time (Equation [2]).

g) were completely wrapped in Whatman #54 paper, inserted into the thimble, and extracted with petroleum ether at condensation rate of 2 to 4 drops per second for 1 h. Wrapped sample residues were removed from the thimble and dried at 60°C overnight.

**Chemical Analyses.** Samples were also ground using a Cyclotech 1093 Sample Mill (Foss Tecator, Hoganas, Sweden) and analyzed for DM (AOAC, 2000; method 930.15), ash (AOAC, 2000; method 942.05), CP (AOAC, 2000; method 990.06), and acid detergent lignin (ADL) using a Daisy II Incubator (Ankom Technology). Solution for ADL was the same as that described in [AOAC, 2000; method 973.18(D)]. The NDR and aNDF were determined using an Ankom Fiber Analyzer (Ankom Technology). Heat stable  $\alpha$ -amylase (number A3306; Sigma-Aldrich Co., St. Louis, MO) was included in all NDR and aNDF procedures (100  $\mu$ L per 0.50 g of sample). Samples were also analyzed for ADIN (Licitra et al., 1996) and EE using the automated Tecator Soxtec System HT6 (Application note AN 301; Foss North America Inc., Eden Prairie, MN).

**In Vitro Digestibility.** This analysis was performed at the University of Nebraska Ruminant Nutrition Laboratory. Two ruminally-cannulated British-breed crossbred steers that were housed in individual stalls were used to collect rumen fluid for determination of in vitro rumen NDR and DM digestibility of all samples. The basal diet consisted of 70% grass hay and 30% concentrate fed twice daily for a total intake of 1.5% of BW. The in vitro disappearance of the samples (1-mm grind) was determined using the method described by Tilley and Terry (1963) but modified by the addition of 1 g per L of urea to the McDougall's buffer (Weiss, 1994). Approximately 5 g of samples were incubated in a Daisy II fermentation vessel (Ankom Technology) in duplicate. Incubation time points for determination of DM and NDF digestibility were 24 and 48 h.

**In Vitro Anaerobic Fermentation and Gas Production.** This analysis was performed at the Ruminant Nutrition Laboratory at the Texas A&M University with product subsamples from the University of Nebraska Ruminant Nutrition Laboratory. The in vitro anaerobic fermentation chamber was similar to that described by Pell and Schofield (1993) and Schofield and Pell (1995). It included an incubator (chamber) with a multi-plate stirrer, pressure sensors attached to the incubation flasks (125-mL Wheaton bottles), an analog to digital converter device, and a PC-compatible computer provided with appropriate software (Pico Technology, Eaton Socon, Cambridgeshire, UK). The pressure inside the flasks was automatically recorded by the computer software every 5 min for 48 h. The ruminal fluid inoculum was obtained from nonlactating, rumen-cannulated Jersey cows, which had free access

to medium-quality mixed forages (mostly grasses) and were fed once daily with a commercial ration for non-lactating cows. The ruminal fluid was filtered through 4 layers of cheesecloth and then through glass wool. The ruminal fluid was mixed continuously with CO<sub>2</sub> to minimize changes in microbial populations and to avoid O<sub>2</sub> contamination. The in vitro medium used was the phosphate-bicarbonate medium and reducing solution of Goering and Van Soest (1970). Feed samples (200 mg) were transferred to a 125-mL Wheaton bottle, which contained a small Teflon-covered stir bar inside. Feed samples were wetted with 2.0 mL of boiled distilled water that had been cooled to room temperature. The water was used to avoid particle dispersion, and discounted by the media. The medium was continuously ventilated with CO<sub>2</sub>. The medium was heated separately to just below boiling temperature and then cooled to room temperature. At this point, cysteine hydrochloride was added. The media pH and CO<sub>2</sub> saturation was controlled by color change of resazurin indicator from purple to pink/colorless, and the optimum pH utilized was between 6.8 and 6.9. Each bottle was filled with 14 mL of this media. Strict anaerobic technique was employed in all transfers (Hungate, 1950; Bryant, 1972). Bottles were closed with previously unused, lightly greased, butyl rubber stoppers, and crimp sealed. All bottles were placed in the fermentation chamber and the respective sensor for each bottle was inserted with a needle. When the fermentation chamber reached 39°C, 4 mL of the filtered mixed ruminal bacteria inoculum was injected into the bottles. The fermentation chamber was closed and when the internal temperature reached 39°C, pressure inside each bottle was zeroed by puncturing the stopper with a needle for 5 s. The fermentation chamber was closed and when the temperature reached 39°C, the recording of the pressure was initiated. The atmospheric pressure was collected at the beginning and at the end of all rounds. After 48 h of fermentation (2,880 data points per sample were collected), each Wheaton bottle was depressurized, the pH and oxidation/reduction (redox) potential were measured, and 40 mL of neutral detergent solution (Van Soest et al., 1991) were added to each Wheaton bottle to determine NDR. Wheaton bottles were crimp sealed and cooked in an autoclave for 60 min at 105°C to determine the undegraded fiber, filtered by gravimetric method using a Whatman 54 filter paper, and dried in an oven. The NDR was determined gravimetrically.

#### Fractional Rate Calculation

The kinetic analysis of the 48-h cumulative gas production was performed using the discrete exponential equation with lag time (Schofield et al., 1994) as shown

in Eq. [1]. All curves were fitted for each feed within substrates using PROC NLIN of SAS (SAS Inst. Inc., Cary, NC) and Gauss-Newton method to obtain the fractional rate of fermentation ( $kf$ ),  $h^{-1}$ .

$$V = \begin{cases} V_F \{1 - \exp[-kf \times (t - \lambda)]\}; & t > \lambda \\ 0; & t \leq \lambda \end{cases} \quad [1]$$

where  $V$  is cumulative gas volume, mL;  $V_F$  is gas volume corresponding to complete matter digestion (asymptote), mL;  $kf$  is fractional rate of fermentation,  $h^{-1}$ ;  $t$  is time, h; and  $\lambda$  is lag time, h.

The fractional rate of disappearance ( $kd$ ) was computed from in vitro digestibility trials assuming first-order kinetics of fermentation and passage. Therefore, fractional rate of disappearance without lag time was computed from in vitro dry matter digestibility (**IVDMD**) data (Table 1) as shown in Eq. [2].

$$kd = \frac{-\ln(1 - \text{in vitro digestibility})}{t} \quad [2]$$

where  $t$  is time, h; and  $kd$  is fractional rate of disappearance,  $h^{-1}$ .

### Modeling Simulations

Monte Carlo simulations were used in the sensitivity analysis of our study. In Monte Carlo simulations, input values are based on probability density functions from which samples are drawn to perform needed calculations and solutions are presented as distributions (Law, 2007). Spearman correlations were used during the simulations to account for non-independency among variables. The sensitivity analysis was obtained from 10,000 iterations and Latin hypercube sampling, which divides the probability density function into intervals of equal probability and from each interval a random sample is obtained (McKay et al., 1979). Distribution fitting was used to obtain the probability density functions of the variables. The goodness of fit of the distribution fitting was assessed by visual distribution and fitting statistics (Chi-squared, Kolmogorov-Smirnov, and Anderson-Darling statistical tests). The determination of most influent variables on the simulation output was accomplished by using standardized regression coefficients (**SRC**; Kutner et al., 2005) and tornado chart. The SRC reflects the change in the standard deviation of the dependent (output) variable associated with one unit change in the standard deviation of the independent (input) variable at a ceteris paribus

condition, i.e., when all other input variables are fixed and unchanged (Helton and Davis, 2002). Monte Carlo simulations, distribution fitting and sensitivity analysis were performed with @Risk v. 5.0 (Palisade Corp., Ithaca, NY).

The TDN for each corn (co)product was predicted using a summative equation similar to that originally proposed by Goering and Van Soest (1970) and modified by Weiss et al. (1992), and used in level 1 solution of the Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 2004; Tedeschi et al., 2005) (Eqs. [3]–[6]). In our model, the digestibility of NDR was computed using fractional rates of fermentation and passage of NDR as shown in Eq. [6]. This modification would provide a more theoretical assessment of ruminal digestibility of the fiber under different scenarios of production. A 20% intestinal digestibility for NDR was assumed to account for large intestine fermentation of fiber (Sniffen et al., 1992), although this coefficient may differ between feeds and should be a subject of future study to improve estimates of total tract fiber digestibility. The  $NE_L$  can be computed using equations provided by the NRC (2001), such as  $NE_L = 0.0245 \times \text{TDN} - 0.12$ .

$$\text{TDN} = 0.98 \times [100 - (\text{NDR} - \text{NDIN}) - \text{CP} - \text{EE} - \text{Ash}] + \text{dCP} + \text{dEE} + \text{dNDR} - 7 \quad [3]$$

$$\text{dCP} = \left[ 1 - 0.004 \times \left( \frac{\text{ADIN} \times \text{CP}}{100} \right) \right] \times \text{CP} \quad [4]$$

$$\text{dEE} = 2.25 \times (\text{EE} - 1) \quad [5]$$

$$\text{dNDR} = \left[ \left( \frac{kf_{\text{NDR}}}{kf_{\text{NDR}} + kp} \right) + 0.2 \right] \times (\text{NDR} - \text{NDIN}) \quad [6]$$

where TDN is apparent total digestible nutrients, percentage of DM; NDR is neutral detergent residue, % DM; NDIN is neutral detergent insoluble nitrogen, % DM; CP is crude protein, percentage of DM; EE is ether extract, percentage of DM; Ash is ash, percentage of DM; dCP is digestible CP, percentage of DM, dEE is digestible EE, percentage of DM; dNDR is ruminal and intestinal digestible NDR, percentage of DM; ADIN is acid detergent insoluble nitrogen, percentage of CP;  $kf_{\text{NDR}}$  is fractional rate of fermentation of NDR,  $h^{-1}$ ;  $kp$  is fractional passage rate,  $h^{-1}$ , and 7 is the estimated TDN metabolic fecal cell soluble.

**Table 2.** Comparison of the dynamics of the anaerobic fermentation of four corn milling (co)products and alfalfa hay for intact feed or neutral detergent and defatted residues<sup>1</sup>

| Variable/Process                                   | n   | Feed <sup>2</sup>   |                     |                    |                     |                     | SEM    | P-value |                  |
|--|-----|---------------------|---------------------|--------------------|---------------------|---------------------|--------|---------|------------------|
|  |     | BRAN                | GERM                | HP-DDG             | HAY                 | BPX                 |        | Feeds   | Run <sup>2</sup> |
| Total gas production (mL per 100 g of DM)          |     |                     |                     |                    |                     |                     |        |         |                  |
| Defatted   | 126 | 32.1 <sup>a</sup>   | 26.4 <sup>b</sup>   | 21.4 <sup>c</sup>  | 16.5 <sup>d</sup>   | 22.9 <sup>c</sup>   | 1.95   | <0.0001 | 0.049            |
| NDR <sup>2</sup>                                   | 139 | 40.1 <sup>a</sup>   | 28.4 <sup>c</sup>   | 26.2 <sup>c</sup>  | 18.1 <sup>d</sup>   | 33.7 <sup>b</sup>   | 1.20   | <0.0001 | —                |
| Intact   | 274 | 24.5 <sup>a</sup>   | 21.1 <sup>b</sup>   | 19.7 <sup>c</sup>  | 20.1 <sup>bc</sup>  | 16.6 <sup>d</sup>   | 0.55   | <0.0001 | 0.0064           |
| Fractional rate of fermentation (h <sup>-1</sup> ) |     |                     |                     |                    |                     |                     |        |         |                  |
| Defatted   | 126 | 0.0812 <sup>b</sup> | 0.0866 <sup>b</sup> | 0.121 <sup>a</sup> | 0.0924 <sup>b</sup> | 0.0817 <sup>b</sup> | 0.0066 | <0.0001 | 0.077            |
| NDR <sup>2</sup>                                   | 139 | 0.0615 <sup>b</sup> | 0.0759 <sup>b</sup> | 0.12 <sup>a</sup>  | 0.106 <sup>a</sup>  | 0.0678 <sup>b</sup> | 0.0058 | <0.0001 | 0.317            |
| Intact   | 274 | 0.17 <sup>b</sup>   | 0.196 <sup>a</sup>  | 0.151 <sup>c</sup> | 0.0935 <sup>d</sup> | 0.165 <sup>bc</sup> | 0.0098 | <0.0001 | 0.005            |
| Lag time (h)                                       |     |                     |                     |                    |                     |                     |        |         |                  |
| Defatted   | 126 | 2.06 <sup>a</sup>   | 1.13 <sup>b</sup>   | 1.16 <sup>b</sup>  | 0 <sup>c</sup>      | 0.73 <sup>bc</sup>  | 0.550  | <0.0001 | 0.049            |
| NDR <sup>2</sup>                                   | 139 | 5.14 <sup>a</sup>   | 2.16 <sup>c</sup>   | 3.08 <sup>b</sup>  | 1.02 <sup>c</sup>   | 3.04 <sup>b</sup>   | 0.265  | <0.0001 | 0.050            |
| Intact   | 274 | 1.18 <sup>c</sup>   | 1.69 <sup>a</sup>   | 1.42 <sup>b</sup>  | 0.97 <sup>c</sup>   | 1.07 <sup>c</sup>   | 0.159  | <0.0001 | 0.040            |
| Estimated DM ruminal digestibility <sup>3</sup>    |     |                     |                     |                    |                     |                     |        |         |                  |
| Fractional <i>kp</i> = 0.04 h <sup>-1</sup>        | 274 | 79.9 <sup>b</sup>   | 82.4 <sup>a</sup>   | 78.2 <sup>c</sup>  | 71.8 <sup>d</sup>   | 79.6 <sup>bc</sup>  | 0.93   | <0.0001 | 0.0044           |
| Fractional <i>kp</i> = 0.06 h <sup>-1</sup>        | 274 | 72.7 <sup>b</sup>   | 75.8 <sup>a</sup>   | 70.8 <sup>c</sup>  | 62.8 <sup>d</sup>   | 72.4 <sup>bc</sup>  | 1.14   | <0.0001 | 0.0044           |
| Fractional <i>kp</i> = 0.08 h <sup>-1</sup>        | 274 | 66.8 <sup>b</sup>   | 70.2 <sup>a</sup>   | 64.6 <sup>c</sup>  | 55.8 <sup>d</sup>   | 66.3 <sup>bc</sup>  | 1.28   | <0.0001 | 0.0044           |

<sup>a-d</sup>Within a row, LSM without a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Values are least squares means (LSM) and SEM is the average of the SE of the LSM.

<sup>2</sup>BRAN = bran, GERM = corn germ dehydrated, HP-DDG and BPX are corn dried distillers grain (co)products in which BPX undergoes a low heat process and HP-DDG has high protein content, HAY = alfalfa hay, Run = fermentation chamber runs as blocking factors in the statistical analysis, and NDR = neutral detergent residue with  $\alpha$ -amylase but without sodium sulfite.

<sup>3</sup>Computed using fractional rate of fermentation of the intact feed. The estimated fractional passage rates (*kp*: 0.04, 0.06, and 0.08 h<sup>-1</sup>) were based on typical diets of dry cows, and low- and high-lactating cows as predicted by the CPM Dairy (Boston et al., 2000).

The NDR was used instead of NDF in Eq. [3]. As discussed before, if sodium sulfite is used in the determination of NDF, a variable amount of neutral detergent insoluble nitrogen (NDIN) will be removed from the residue, depending on the feed. Therefore, we suggest the use of NDR (without sodium sulfite) adjusted for NDIN. If NDF (with sodium sulfite) is used instead, no adjustment for NDIN should be performed because it would underpredict the fiber content of the feed.

To evaluate the potential impact of these changes in animal performance, modeling simulations were performed with the Cornell-Penn-Miner (CPM) Dairy model (Boston et al., 2000; Tedeschi et al., 2008; <http://www.cpm-dairy.com/Index.html>) using typical dairy cow diets. The evaluation of the simulations was performed as described by Tedeschi (2006).

### Statistical Analysis

**In Vitro Anaerobic Fermentation and Gas Production.** The statistical analysis was performed using the PROC MIXED of SAS (SAS Inst. Inc.), assuming an incomplete block design (IBD). The computerized anaerobic fermentation chamber can analyze 22 Wheaton bottles at the same time; therefore, several runs (batches) were conducted to process all the samples. Each run was considered a block. Two analyses were conducted: 1) the corn milling (co)products and

the alfalfa hay were considered the treatments (Table 2) and 2) the intact feed, NDR, and DFR (substrates) were considered the treatments (Table 3). The interaction between feeds and substrate was of no interest, and therefore, was not evaluated. Treatments were considered fixed effects in these analyses and blocks (runs) were assumed to be random. The interaction between treatment and block was not considered. The variance component type was assumed for the variance-(co)variance matrix. The least squares means were used to compare treatments. The statistical model is shown below.

$$Y_{ijk} = \mu + \text{TRT}_i + \text{BLOCK}_j + e_{ijk}$$

where  $\mu$  is the overall mean,  $\text{TRT}_i$  is the  $i$ th treatment,  $\text{BLOCK}_j$  is the  $j$ th fermentation run, assuming identically, independently distributed  $\sim N(0, \sigma_{\text{Block}}^2)$ , and  $e$  is the uncontrolled, random error, assuming identically, independently distributed  $\sim N(0, \sigma^2)$ .

**Modeling Regression.** The chemical composition and digestibility of corn (co)products were used as independent variables of the multiple regressions to predict the NDR fractional fermentation rate ( $kf_{\text{NDR}}$ ). Three steps were followed. In the first step, the PROC REG of SAS (SAS Inst. Inc.) was used with the STEPWISE selection criterion to identify most influential

**Table 3.** Comparison of the dynamics of the anaerobic fermentation of intact feed, neutral detergent and defatted residues for four corn milling (co)products and alfalfa hay<sup>1</sup>

| Variables <sup>2</sup>                             | n   | Substrates         |                     |                     | SEM    | P-value    |       |
|--|-----|--------------------|---------------------|---------------------|--------|------------|-------|
|  |     | Intact             | NDR <sup>2</sup>    | Defatted            |        | Substrates | Run   |
| Total gas production (mL per 100 g of DM)          |     |                    |                     |                     |        |            |       |
| Alfalfa hay (standard feed)                        | 37  | 19.8               | 18.0                | 18.3                | 1.080  | 0.380      | 0.307 |
| Bran   | 138 | 24.5 <sup>c</sup>  | 40.6 <sup>a</sup>   | 31.7 <sup>b</sup>   | 0.911  | <0.0001    | 0.085 |
| Germ dehydrated                                    | 117 | 20.9 <sup>c</sup>  | 27.6 <sup>a</sup>   | 25.5 <sup>b</sup>   | 0.873  | <0.0001    | 0.025 |
| HP-DDG   | 124 | 19.5 <sup>b</sup>  | 26.2 <sup>a</sup>   | 21.4 <sup>b</sup>   | 0.915  | <0.0001    | 0.342 |
| BPX  | 123 | 16.4 <sup>c</sup>  | 34.7 <sup>a</sup>   | 22.9 <sup>b</sup>   | 0.891  | <0.001     | 0.057 |
| Fractional rate of fermentation (h <sup>-1</sup> ) |     |                    |                     |                     |        |            |       |
| Alfalfa hay (standard feed)                        | 37  | 0.116 <sup>a</sup> | 0.104 <sup>ab</sup> | 0.0847 <sup>b</sup> | 0.0069 | 0.0062     | 0.182 |
| Bran   | 138 | 0.171 <sup>a</sup> | 0.04 <sup>b</sup>   | 0.0549 <sup>b</sup> | 0.0108 | <0.0001    | 0.024 |
| Germ dehydrated                                    | 117 | 0.192 <sup>a</sup> | 0.0558 <sup>b</sup> | 0.0697 <sup>b</sup> | 0.0097 | <0.0001    | 0.027 |
| HP-DDG   | 124 | 0.151 <sup>a</sup> | 0.101 <sup>b</sup>  | 0.105 <sup>b</sup>  | 0.0088 | <0.0001    | 0.019 |
| BPX  | 123 | 0.167 <sup>a</sup> | 0.044 <sup>b</sup>  | 0.0622 <sup>b</sup> | 0.0095 | <0.0001    | 0.028 |
| Lag time (h)                                       |     |                    |                     |                     |        |            |       |
| Alfalfa hay (standard feed)                        | 37  | 1.26 <sup>a</sup>  | 1.18 <sup>a</sup>   | 0.26 <sup>b</sup>   | 0.24   | 0.0104     | 0.215 |
| Bran   | 138 | 1.09 <sup>c</sup>  | 5.10 <sup>a</sup>   | 1.81 <sup>b</sup>   | 0.32   | <0.0001    | 0.015 |
| Germ dehydrated                                    | 117 | 1.59 <sup>a</sup>  | 1.87 <sup>a</sup>   | 0.70 <sup>b</sup>   | 0.15   | <0.0001    | 0.022 |
| HP-DDG   | 124 | 1.41 <sup>b</sup>  | 2.60 <sup>a</sup>   | 0.68 <sup>c</sup>   | 0.26   | <0.0001    | 0.012 |
| BPX  | 123 | 1.12 <sup>b</sup>  | 2.77 <sup>a</sup>   | 0.25 <sup>c</sup>   | 0.22   | <0.0001    | 0.011 |

<sup>a-c</sup>Within a row, LSM without a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Values are least squares means (LSM) and SEM is the average of the SE of the LSM.

<sup>2</sup>BRAN = bran, GERM = corn germ dehydrated, HP-DDG and BPX are corn dried distillers grain (co)products in which BPX undergoes a low heat process and HP-DDG has high protein content, NDR = neutral detergent residue with  $\alpha$ -amylase but without sodium sulfite.

variables (reduced model). During this step, influential points and outliers were removed if their studentized residual was outside of  $-2.5$  and  $2.5$  (Kutner et al., 2005). In the second step, the selected variables and their second order interactions were evaluated in the PROC GLM of SAS (SAS Inst. Inc.) in a stepwise fashion to identify variables and their interaction (full model) that would maximize the  $R^2$  and minimize Mallows' Cp (McNeil, 1983). During this step, influential points and outliers previously removed in the first step were added to the model and re-assessed. This process was necessary because the full model might account for some outliers previously removed in the reduced model. In the third step, the final regression was devised using the PROC REG using the selected full model and the variance-(co)variance matrix ( $s^2\{b\}$ ) was obtained. The unbiased estimator of the mean response ( $\hat{Y}_h$ ) and variance ( $s^2\{\hat{Y}_h\}$ ) was determined using Equations [7] and [8], respectively (Kutner et al., 2005). The  $s^2\{b\}$  can be used to compute the confidence (CI) and prediction (PI) intervals of future predictions.

$$\hat{Y}_h = X_h' \beta \quad [7]$$

$$s^2\{\hat{Y}_h\} = X_h' \cdot s^2\{b\} \cdot X_h \quad [8]$$

where  $\hat{Y}_h$  is the predicted mean value,  $X_h'$  is the (transposed) vector of the values of independent variables used in the full model regression,  $\beta$  is the vector of coefficients of the full model regression,  $s^2\{\hat{Y}_h\}$  is the variance of the predicted mean value,  $s^2\{b\}$  is the variance-(co)variance matrix of the estimated coefficients of the full model regression.

The predicted  $kf_{\text{NDR}}$  values were used in Eq. [6] to compute digestible NDR with the Monte Carlo simulation. If predicted values were negative, they were assumed to be zero for simulation purposes.

## RESULTS AND DISCUSSION

Chemical composition of corn milling (co)products is listed in Table 1. The current CPM-Dairy feed library lists the CP, EE, ash, NDF, ADF, ADIN, and ADL concentration of corn distillers grains (with solubles) to be 30.3, 14.5, 5.89, 32.2, 17.8, 5.16, and 4.6% of DM basis. As expected, these values are similar to those observed on samples of BPX, but ADIN was lower (2.97% of DM). This observation was expected, given that the BPX samples are a product of a dry milling process in which the mash is spared from heating, thereby reducing the likelihood of heat-damaged protein. The remaining BRAN, GERM, and HP-DDG components are produced from a separate process in



which the BRAN and GERM are removed from the corn kernel before fermentation. Consequently, the HP-DDG product resulting after fermentation is more concentrated in protein (CP = 44.6%). Given that the dry milling process utilizes a larger proportion of starch, the observed level of NDR in these (co)products was large. The concentration of EE in the BRAN, GERM, HP-DDG, and BPX is listed in Table 1. Because a high concentration of lipid is located in the germ of the corn kernel the high concentration in GERM was expected (Stock et al., 1999). The *in vitro* DM and NDF digestibility (24 and 48 h) for all feeds are listed in Table 1. Observations of 24 and 48 h NDF digestibility were all high suggesting the fiber portion of all feeds is highly digestible and similar to that observed by Miron et al. (2001) and Getachew et al. (2004).

The IVDMD at 24 and 48 h of incubation decreased exponentially as ADIN increased (Figure 1). The degree of IVDMD reduction was more accentuated at 24 than at 48 h of incubation as shown by their fractional rates ( $-1.105$  vs.  $-1.027$  h<sup>-1</sup>, respectively), suggesting that at earlier times of incubation ADIN had a greater influence on the IVDMD than at later times. Individual corn (co)products had different relationships between ADIN and IVDMD, likely because they have distinctly different contents of CP and ADIN (Table 1) as well as different extents and degree of heat damage. The IVDMD of HP-DDG was essentially not affected by levels of ADIN as much as BRAN, GERM, and BPX were for both 24 and 48 h of incubation (Figure 1). The ADIN is known to linearly affect the digestibility of forage CP ( $r = 0.93$ ; Yu and Thomas, 1976) and nonforage CP ( $r = 0.81$ ; Nakamura et al., 1994), but there is evidence that ADIN is not completely indigestible, and about 60% of ADIN is digestible in distillers grains (Van Soest, 1994). Van Soest (1994) suggested that ADIN in distillers grains might have a different behavior than ADIN in silages and ammoniated forages. Van Soest (1994) postulated that one of the following assumptions or a combination might occur when distillers grains are fed to ruminants: 1) there is a true digestibility of ADIN, 2) metabolic fecal N is reduced, and 3) part of ADIN is absorbed from the small intestine but it is excreted in the urine.

The BRAN produced more ( $P < 0.0001$ ) gas than the other corn milling (co)products and HAY, regardless of the substrate (intact, NDR, or DFR) used for anaerobic fermentation (Table 2). The HP-DDG consistently had the lowest total gas production ( $P < 0.0001$ ) among the corn (co)products. Compared with HAY, the rate of fermentation was faster for all corn milling (co)products, and GERM had the fastest rate of fermentation for the intact substrate. Consistently, the DM ruminal digestibility using passage rates ( $kp$ ) of CPM Dairy

simulations for dry cows (0.04 h<sup>-1</sup>), and low- (0.06 h<sup>-1</sup>) and high-producing (0.08 h<sup>-1</sup>) cows indicated that GERM would have the greatest ruminal degradability; BRAN and BPX would have similar values, but HP-DDG would be the corn milling (co)product with the lowest ruminal degradability (Table 2). Kleinschmit et al. (2007) evaluated ruminal degradation and intestinal digestion of DDGS and reported differences in RUP among 5 different sources of DDGS. As shown in Table 2, CPM Dairy simulations predicted that the DM ruminal degradation varied from 78.1 to 82.3% for dry cows ( $kp = 0.04$  h<sup>-1</sup>), 70.5 to 75.7% for low- ( $kp = 0.06$  h<sup>-1</sup>), and 64.3 to 70.1% ( $kp = 0.08$  h<sup>-1</sup>) for high-producing lactating cows based on the fractional rate of fermentation of the intact feed. High-producing lactating cows would have the largest difference (70.1 to 64.3%) of DM digestibility of these corn (co)products.

Table 3 contains the total gas production, fractional rate of fermentation, and lag time of the intact feed, NDR, and DFR for HAY and the 4 corn milling (co)products. In general, the total gas production (mL per 100 g of DM) of the corn (co)products was greater for the NDR than the DFR, which was greater than the intact feed substrate. This finding confirms that EE compounds are not fermented under anaerobic conditions, therefore providing no support for ruminal microbial growth (Nagaraja et al., 1997). The intact and DFR substrates of HP-DDG were not different ( $P < 0.05$ ). The fractional rate of fermentation was faster for intact than for either NDF or DFR, likely because these samples had a greater concentration of nonfiber carbohydrates that ferment more rapidly than fiber (Miron et al., 2001). In addition, the defatted residue had a lower lag time than either the intact or NDR substrates likely because the chemical treatment affected the physical structure of the feed and reduced the toxic effect of lipids, easing the attachment and access by the microbes. In the case of corn (co)products this was expected because these samples were higher in fat, which is known to affect microbial digestion of feeds by limiting the number of microbial attachment sites on the feed particle (McAllister et al., 1994).

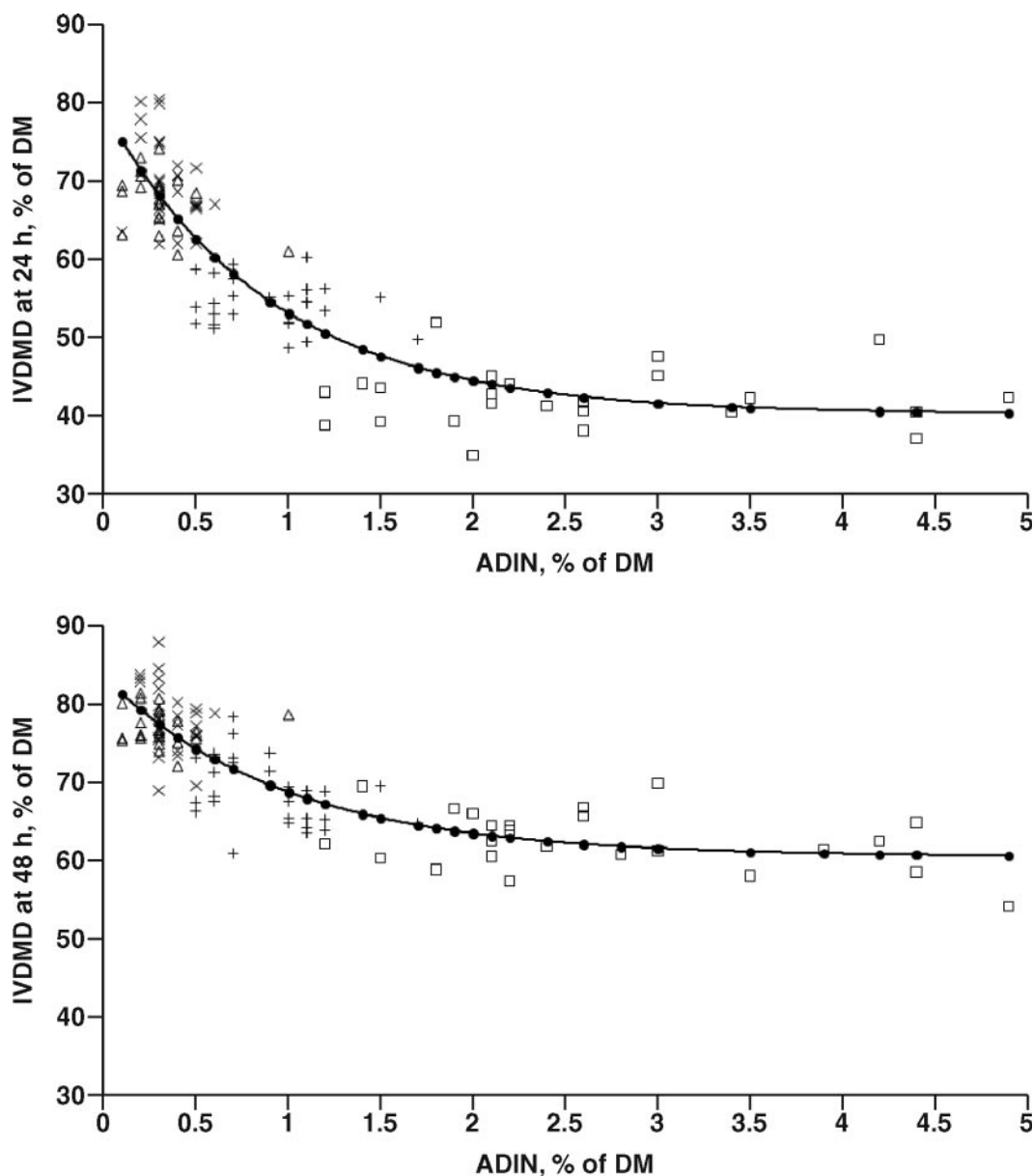
Table 4 lists the distribution statistics and equation for the fractional rate of fermentation of each corn (co)product and substrates. As expected, fractional rates were lower for DFR and NDR, except for HP-DDG. In general, distributions did not follow a normal distribution. Based on the mean, mode, and fitted distributions the fractional fermentation rate of HP-DDG and BPX might vary considerably. These results may indicate lower consistency of presence of fermentable material or substances that inhibit fermentation among batches. The regression of the fractional rate of fermentation of the feeds on the feed identification (not

shown) suggested that fractional rate of fermentation changed across time within corn (co)products. Despite the distributions, the fractional rates of fermentation for NDR and DFR listed in Table 4 were similar to those reported by Varga and Hoover (1983) for distillers grains (0.072 h<sup>-1</sup>) as well as the CPM Dairy listed estimate (0.07 h<sup>-1</sup>). Ideally, more samples should be evaluated to confirm the distribution shape of these corn (co)products and evaluate the factors affecting the quadratic relationship between the fractional fermentation rate and time of sample collection.

**Modeling Simulations**

Equations to predict  $kf_{NDR}$  from feed chemical composition and digestibility were developed for each corn (co)product. Equation [9] was developed for BRAN with  $n = 20$ , and had  $R^2 = 0.803$  and root of mean square error (RMSE) = 0.00695 h<sup>-1</sup>.

$$\text{BRAN } kf_{NDR} = -0.231 + 0.00524 \times \text{aNDF} + 0.0128 \times \text{CP} - 0.00628 \times \text{ADL}, \quad [9]$$



**Figure 1.** Scatter plot of in vitro DM digestibility (IVDMD) at 24 (A) or 48 h (B) and ADIN of corn bran ( $\Delta$ ), high protein dried distillers grains (HP-DDG;  $\square$ ), and DDGS with low heat during the drying process (BPX;  $+$ ). Predicted equation ( $\bullet$ ) is  $\text{IVDMD} = 40.11 + 38.76 \times \text{Exp}(-1.105 \times \text{ADIN})$  with  $n = 102$  and mean square error of 5.39% for A and  $\text{IVDMD} = 60.44 + 22.92 \times \text{Exp}(-1.027 \times \text{ADIN})$  with  $n = 104$  and mean square error of 3.97% for B.

**Table 4.** Distribution statistics of the fractional fermentation rate of intact feed, neutral detergent and defatted residues for four corn milling (co)products and alfalfa hay

| Item <sup>1</sup> | n  | Mean<br>(h <sup>-1</sup> ) | Mode<br>(h <sup>-1</sup> ) | SD<br>(h <sup>-1</sup> ) | 90% Confidence interval     |                             | Distributions <sup>2</sup>                |
|-------------------|----|----------------------------|----------------------------|--------------------------|-----------------------------|-----------------------------|---|
|                   |    |                            |                            |                          | Lower<br>(h <sup>-1</sup> ) | Upper<br>(h <sup>-1</sup> ) |   |
| Intact feed       |    |                            |                            |                          |                             |                             |   |
| BRAN              | 31 | 0.159                      | 0.151                      | 0.0455                   | 0.102                       | 0.272                       | Weibull (1.54, 0.0767, +0.0902)           |
| GERM              | 30 | 0.184                      | 0.166                      | 0.0406                   | 0.12                        | 0.265                       | LogNormal (1.28, 0.0406, -1.095)          |
| HP-DDG            | 29 | 0.149                      | 0.113                      | 0.0297                   | 0.113                       | 0.198                       | Logistic (0.147, 0.0179)                  |
| BPX               | 30 | 0.16                       | 0.157                      | 0.0377                   | 0.107                       | 0.263                       | Weibull (1.46, 0.06, +0.105)              |
| NDR               |    |                            |                            |                          |                             |                             |   |
| BRAN              | 22 | 0.0635                     | 0.0642                     | 0.0195                   | 0.0334                      | 0.127                       | Logistic (0.0618, 0.0101)                 |
| GERM              | 22 | 0.0803                     | 0.0496                     | 0.0226                   | 0.0475                      | 0.132                       | Logistic (0.0786, 0.0128)                 |
| HP-DDG            | 21 | 0.114                      | 0.123                      | 0.0367                   | 0.0161                      | 0.18                        | Weibull (17.5, 0.529, -0.399)             |
| BPX               | 23 | 0.0695                     | 0.076                      | 0.0346                   | 0.0346                      | 0.129                       | Logistic (0.0684, 0.00801)                |
| DFR               |    |                            |                            |                          |                             |                             |   |
| BRAN              | 25 | 0.0852                     | 0.0835                     | 0.0209                   | 0.0420                      | 0.129                       | BetaGeneral (4.017, 3.534, 0.0197, 0.143) |
| GERM              | 19 | 0.0914                     | 0.0844                     | 0.0141                   | 0.0643                      | 0.119                       | LogLogistic (0.0145, 0.0757, 9.43)        |
| HP-DDG            | 19 | 0.117                      | 0.109                      | 0.0150                   | 0.0952                      | 0.144                       | LogLogistic (0.0898, 0.0228, 2.97)        |
| BPX               | 18 | 0.0844                     | 0.0833                     | 0.0149                   | 0.0610                      | 0.14                        | Logistic (0.0825, 0.00539)                |

<sup>1</sup>BRAN = bran, GERM = germ dehydrated, HP-DDG and BPX are corn dried distillers grain (co)products in which BPX undergoes a low heat process and HP-DDG has high protein content, NDR = neutral detergent residue with α-amylase but without sodium sulfite, and DFR = defatted residue.

<sup>2</sup>For LogNormal distributions, the values are parameters for mean and SD. For Logistic and LogLogistic, the values are parameters for continuous location, continuous scale, and continuous shape, respectively. For Weibull, the values are parameters for continuous shape and continuous scale, respectively. For BetaGeneral, the values are parameters for two continuous shapes and minimum and maximum boundaries, respectively. The distribution shift is represented by the + or - signs, indicating that distribution shifts to the right or left, respectively. Distribution fitting was obtained with @Risk v. 5.0 (Palisade Corp., Ithaca, NY).

where  $kf_{NDR}$  is the fractional rate of fermentation of NDR, h<sup>-1</sup>; aNDF is neutral detergent fiber, % of DM; CP is crude protein, % of DM; and ADL is as % of DM.

Equation [10] was developed for GERM with n = 18, and had R<sup>2</sup> = 0.879 and RMSE = 0.00793 h<sup>-1</sup>.

$$\text{GERM } kf_{NDR} = -0.189 + 0.0146 \times \text{ADIN}_{CP} + 0.00267 \times \text{IVDMD}_{24h} + 0.017 \times \text{ADL}, \quad [10]$$

where  $kf_{NDR}$  is the fractional rate of fermentation of NDR, h<sup>-1</sup>; ADL is as % of DM; ADIN<sub>CP</sub> is acid detergent insoluble nitrogen, % of CP; and IVDMD<sub>24 h</sub> is IVDMD at 24 h, %.

For HP-DDG, Eq. [11] was developed with n = 20, and had R<sup>2</sup> = 0.806, and RMSE = 0.00806 h<sup>-1</sup>.

$$\begin{aligned} \text{HP-DDGS } kf_{NDR} = & 10.6 - 0.111 \times \text{DM} \\ & - 0.00393 \times \text{NDR} - 2.68 \times \text{EE} + 0.0952 \times \text{ADL}_{NDR} \\ & + 0.0289 \times \text{DM} \times \text{EE} - 0.00112 \times \text{DM} \times \text{ADL}_{NDR}, \end{aligned} \quad [11]$$

where  $kf_{NDR}$  is the fractional rate of fermentation of NDR, h<sup>-1</sup>; DM is as % as fed; NDR is neutral detergent

residue, % of DM; EE is ether extract, % of DM; and ADL<sub>(NDR)</sub> is ADL, % of NDR.

Equation [12] was developed for BPX with n = 20, and had R<sup>2</sup> = 0.877 and RMSE = 0.00507 h<sup>-1</sup>.

$$\begin{aligned} \text{BPX } kf_{NDR} = & 18.9 - 0.207 \times \text{DM} - 1.64 \times \text{EE} \\ & - 0.0181 \times \text{Ash} + 0.0208 \times \text{ADIN} \\ & + 0.0025 \times \text{IVNDFD}_{24h} + 0.0179 \times \text{DM} \times \text{EE}, \end{aligned} \quad [12]$$

where  $kf_{NDR}$  is the fractional rate of fermentation of NDR, h<sup>-1</sup>; DM is as % as fed; EE is ether extract, % of DM; Ash is as % of DM; ADIN is acid detergent insoluble nitrogen, % of DM; and IVNDFD<sub>24 h</sub> is in vitro NDF digestibility at 24 h, %.

Despite the small sample size (n ≤ 20), the predictability of  $kf_{NDR}$  was greater for BRAN, GERM, and BPX than for HP-DDG as shown by the greater R<sup>2</sup>. A major application of summative equations is to recognize the cause and effect relationships between chemical components and availability (Van Soest, 1967). The expected feed characteristics such as in vitro digestibility, fiber, ether extract, CP, and ash were the most influential variables in predicting the  $kf_{NDR}$ . The form of protein was important in determining the  $kf_{NDR}$  for BRAN, GERM, and BPX, but not for HP-DDG, which

had the greatest CP content (Table 1). The interaction between DM and EE was significant for HP-DDG and BPX, suggesting that  $kf_{\text{NDR}}$  was lower for feeds with greater values of EE and the  $kf_{\text{NDR}}$  declined faster when EE was greater and DM increased. This is in agreement with evidence that fat reduces digestibility of fibrous matter under in vitro anaerobic fermentation conditions (Chalupa et al., 1984).

Simulations for TDN were obtained by combining Eqs. [3]–[6] and [9]–[12]. Distribution and Spearman correlations for variables used in Eqs. [9]–[12] were obtained (not shown) and used to compute the TDN distribution for BRAN, GERM, HP-DDG, and BPX. The predicted digestible NDR (Eq. [6]) used a passage rate of  $0.06 \text{ h}^{-1}$ . BRAN had a normal-like distribution, GERM had a Weibull shape distribution, and HP-DDG and BPX were more skewed. For BRAN and GERM, EE was the variable with the highest influence on the TDN value (greatest SRC value). For each SD change in EE, TDN of BRAN and GERM would vary by 0.52 and 0.67 SD units, respectively. For instance, if EE in the GERM would increase by 1.7 units (this is the SD of EE for GERM; Table 1), the TDN of GERM would increase by approximately 2% units ( $0.67 \times 3.2$ ; 3.2 is the SD of TDN for GERM), assuming a linear relationship between these variables and that all other variables would remain at their mean value (*ceteris paribus* condition). Therefore, controlling for influential variables is critical to ensure adequate predictions of nutritional values of feeds during the manufacturing phase of the (co)products.

For HP-DDG, most influential variables (ADL, NDR, and EE) had negative effect on TDN, indicating that little improvement on HP-DDG TDN is possible, assuming the present composition. For BPX, increasing the in vitro NDF digestibility at 24 h would increase BPX TDN likely due to its positive effect on  $kf_{\text{NDR}}$ . The most influential variables of HP-DDG and BPX were the same that affected the values of  $kf_{\text{NDR}}$ . Thus, fiber quality is essential to ensure higher TDN values of these feeds. Previous research has determined that both BRAN and GERM contain larger proportions of nonfiber carbohydrates (Kelzer et al., 2007) than HP-DDG and BPX. In fact, assuming a normal distribution for  $kp$  (mean =  $0.06 \text{ h}^{-1}$  and SD =  $0.006 \text{ h}^{-1}$ ),  $kp$  negatively impacted the predicted TDN for all corn (co)products to different degrees. The SRC value of  $kp$  for BRAN, GERM, HP-DDG, and BPX was  $-0.27$ ,  $-0.25$ ,  $-0.17$ , and  $-0.29$ , respectively. This would result in a reduction in the mean value of TDN by 0.57, 0.82, 0.91, and 0.88 percentage units; respectively, so,  $kp$  could slightly decrease TDN by up to 1 percentage unit of their mean value. Therefore, the majority of the change in the TDN value would come from feed composition

assuming a mean value of  $0.06 \text{ h}^{-1}$  for  $kp$ . These findings support the necessity of using stochastic programming to formulate diets for dairy cattle as suggested by Tozer (2000) and the need to establish protocols of quality control processes for feed sampling to minimize the impact of nutrient variation on milk production and economic return (St-Pierre and Cobanov, 2007).

The 90% CI values of TDN were [90, 96], [96, 105], [82, 94], and [89, 96] for BRAN, GERM, HP-DDG, and BPX with a  $kp$  of  $0.04 \text{ h}^{-1}$  and [86, 93], [89, 101], [76, 89], and [84, 92] with a  $kp$  of  $0.08 \text{ h}^{-1}$ , respectively. As expected, increasing  $kp$  from  $0.04$  to  $0.08 \text{ h}^{-1}$  would decrease TDN values for all corn (co)products. The BRAN (co)product was the most resistant to changes in  $kp$ . The 90% CI indicated that HP-DDG was the product with the most variable TDN (broader CI) and BRAN was most consistent product (narrower CI). The reduction in the upper value of the 90% CI for BPX was the greatest whereas the reduction in the lower value of the 90% CI of GERM and HP-DDG were the greatest. This suggests that GERM and HP-DDG are more prone to have decreased TDN value when  $kp$  increases either by increased DMI, changes in the diet constituents, or BW (Seo et al., 2006). Based on these simulations, when energy (i.e., TDN) is first limiting for high-producing animals, BRAN and GERM would be recommended because the probability of obtaining a product with greater consistency and greater energy values than HP-DDG or BPX. Conversely, the inclusion of HP-DDG may be considered when protein is more limiting.

Based on the distillers grain samples used in this study, our findings suggested that the proportion of fiber digested in the rumen was affected by the degree of sample processing and fat removal. When compared with the values currently used in the feed dictionary of the CPM Dairy, our findings suggested the reported values for the rate of NDF digestion are indicative of many corn (co)products; however, these estimates vary greatly across different types of corn (co)products. Our simulations of TDN values demonstrated that differences in fermentability and chemical composition of these corn (co)products might considerably affect the supply of energy for lactating dairy cows, assuming a high correlation between TDN and  $\text{NE}_L$ . Feeding strategies should be adopted to select appropriate corn (co)products for animal groups based on the demand of energy and protein, and economical risks associated with changes in milk production.

This research may be applied to routine ration formulation procedures that should improve the accuracy of predicting nutrient supply and utilization of animals consuming diets containing corn milling (co)products. In addition, the analytical methods developed in this

study may serve as a valuable tool to assess nutrient quality and uniformity when samples differ in chemical composition.

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