DETERMINING FIBER AND PROTEIN DEGRADATION RATES OF CORN MILLING (CO)PRODUCTS AND THEIR EFFECTS ON RUMEN BACTERIAL POPULATIONS AND LACTATING DAIRY COW PERFORMANCE

A Thesis

by

WHITNEY LYNN WILLIAMS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2011

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Luis O. Tedeschi
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ABSTRACT

Determining Fiber and Protein Degradation Rates of Corn Milling (Co)Products and Their Effects on Rumen Bacterial Populations and Lactating Dairy Cow Performance.

(May 2011)

Whitney Lynn Williams, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Luis O. Tedeschi

Corn milling (co)products (n=120) were evaluated for their neutral detergent fiber residue (**NDR**) and neutral detergent insoluble protein (**NDIP**) ruminal degradation rates using several in vitro methods. Two (co)products (**BPX-DDGS** and **HP-DDG**) were fed to lactating dairy cows (n=44) to evaluate effects on milk production. The Cornell-Penn-Miner Institute (**CPM**) Dairy model was used to formulate diets and predict milk production. In vitro determined NDR and NDIP rates and were compared to CPM-dairy feed library values, and model predictions were compared with observed milk production. Additionally, BPX-DDGS and HP-DDG were defatted and compared with their intact forms for fermentation characteristics using the in vitro gas production (**IVGP**) technique. Fermentations were analyzed for rumen bacterial population shifts using the 16S rDNA bacterial tag encoded FLX amplicon pyrosequencing (**bTEFAP**) technique. Lastly, a novel ruminal in vitro method was described to measure the soluble protein fraction of feeds, with adjustments for microbial contamination.

Fermentation rate of the NDR of BPX-DDGS and HP-DDG (0.08 and 0.07 h^{-1}, respectively) and NDIP degradation rates (0.07 and 0.06 h^{-1}, respectively) were similar to CPM-dairy feed library NDR and NDIP rates of corn distillers grain (0.07 and 0.05 h^{-1}, respectively). Model predictions using standard and in vitro determined values did not differ. As BPX-DDGS decreased and HP-DDG increased in the diet, observed milk production tended to decrease linearly (P = 0.08). There was a cubic effect for milk fat % (P = 0.03) and a cubic trend for milk fat yield (P = 0.09). Milk protein yield also tended to decrease linearly (P = 0.06). CPM-dairy model prediction accuracies were less than 50 %. Defatting (co)products reduced lag time and fractional rate of fermentation by at least half for BPX-DDG, and had no effect on HP-DDG. Defatting both (co)products increased the fibrolytic (26.8 to 38.7 %) and proteolytic (26.1 to 37.2 %) bacterial guild populations and decreased the lactate-utilizing bacterial guild (3.06 to 1.44 %). The novel ruminal in vitro method determined that the specific activity of ammonia production was not different among (co)products. However, results were within numerical range of previously used methodologies.

DEDICATION

This thesis is dedicated to my family. Since I was a little girl you told me I could grow up to be anything I wanted to be, and I believed that without question. Therefore, at the age of five, I logically concluded that I was destined to be a famous country singer. However, in my teen years, I realized next to singing, another passion in my life was agriculture. The trials and tribulations I experienced on the ranch resulted in ethics, self-reliance, pride and a love for cattle. Now that I am receiving my M.S. degree in Animal Science, I look back on all the milestones of my life, and realize all that I have accomplished could not have been possible without your love and support. I can't tell you enough how much I appreciate your praise for a job well done, the discipline when I was out of line, your faith when I was uncertain, and your strength and encouragement when I felt defeated and wanted to give up. I hope you realize that with every accomplishment, my competitive nature was not the only thing driving me, and that I will probably never stop trying to make you proud. YKIWALY.

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CHAPTER I

INTRODUCTION

In the last decade, the ethanol production industry has exploded in the Midwest, in the pursuit to lessen the United States' dependence on foreign oil and to endorse clean burning biofuels. This rapid growth has resulted in a number of corn milling (co)products from corn grain processing that are suitable for livestock consumption. As the demand for ethanol production drives corn prices skyward, cost of milk production follows, as corn is the primary energy concentrate in dairy diets. However, corn milling (co)products may serve as a less expensive alternative to corn to be included in the diets of lactating dairy cattle.

During ethanol production, after starch removal, the remaining corn constituents are concentrated three-fold in what is called stillage. The stillage is then further processed and refined into nutrient dense corn (co)products. These corn milling (co)products are an excellent source of CP, specifically RUP, and digestible fiber for ruminant consumption. Ideally corn milling (co)products are a viable energy and protein source for ruminant diets with high nutrient demand, such as with lactating dairy cattle. However, processing methods vary from plant to plant as well as within a single ethanol plant, rendering industry wide inconsistency of the nutrient content and bioavailability among (co)products. Specifically, (co)products are known to have varying high levels of unsaturated fatty acid content which may impair fiber digestion, resulting in decreased

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This thesis follows the style and form of the Journal of Dairy Science.

absorption of acetate, and subsequently hinder milk fat synthesis in lactating dairy cattle. Hence, uncertainty in nutrient value has limited the use of corn milling (co)products in dairy cow rations to typically around 10 % (DM basis) of the ration.

Since the potential of corn milling (co)products has been reported and technology has evolved, ethanol producers are moving to improve the efficiency and consistency of their processing methods. Consequently, there is an urgent need to accurately characterize the corn milling (co)products of novel processing techniques, before they can successfully be included in ruminant rations at higher levels. Therefore, it is important to develop accurate techniques that describe the nutrient content and availability of these novel corn milling (co)products to provide more precise chemical input values for nutrition models. Determining the biological value of corn milling (co)products will not only lead to more precise ration balancing but also to increased buyer confidence.

As part of a multiphase research assignment, the objective of this work was to re-evaluate and finalize the development of a feed evaluation system developed to describe the nature of the variability of the nutritionally significant components of corn milling (co)products by: (1) increasing the sample size previously analyzed, (2) adding protein fractionation and determination of degradation rates for each key fraction , and (3) conducting a lactation trail to evaluate accuracy and precision of the Cornell Net Carbohydrate and Protein System and the Cornell-Penn-Miner Institute Dairy model predictions and the calibration of these systems to use Poet LLC (Sioux Falls, SD) corn milling (co)products. Additionally, the incorporation of a novel technique that identifies

rumen bacterial DNA was evaluated and compared with in vitro gas production, to help describe the fermentation dynamics of intact or modified (de-oiled) (co)products.

CHAPTER II

EFFECTS OF FEEDING TWO CORN DISTILLERS' GRAINS ON LACTATING DAIRY COW PERFORMANCE AND EVALUATION OF THE CPM-DAIRY MODEL PREDICTIONS

INTRODUCTION

The use of corn for ethanol production has resulted in a number of corn milling (co)products that are available for livestock consumption. Corn (co)products are typically a good source of energy and protein, due to the concentrating effects of the milling process. Nutrient dense feedstuffs like corn (co)products are, therefore, viable ingredients to be used in the diets of lactating dairy cows. Studies have reported favorable results on milk production and quality (Powers et al., 1995; Nichols et al., 1998) when corn distillers' grain plus solubles (**CDGS**) were included at 15 to 20 % of the ration. Janicek et al. (2008) also reported greater DMI, milk yield, milk protein and fat yields when CDGS was included at 30 % of the diet.

However, it is known that nutritional composition of corn milling (co)products can vary from plant to plant (Spiehs et al., 2002). Therefore, chemical analyses of (co)products are essential prior to ration formulation. The Cornell-Penn-Miner Institute (**CPM)** Dairy model offers a custom feed dictionary that includes chemical profiles of commonly used feedstuffs that may be altered to fit the user's needs. Hence, profiles can be calibrated to reflect the chemical analysis of a feed, to give more accurate animal performance predictions. Common laboratorial chemical analyses, however, do not

determine fractional ruminal degradation rates of the potentially-degradable feed fractions. Additionally, although corn (co)products are a good source of energy and protein, they may vary considerably in their nutrient availability (Tedeschi et al., 2009). It would be advantageous to know the degradation rates of key nutrient fractions that are known to be variable across feeds and are not routinely measured; specifically, the neutral detergent fiber (**NDF**) and neutral detergent insoluble protein (**NDIP**) fractions (Sniffen et al., 1992; Schwab et al., 2003). Including more accurate degradation rates of feedstuffs may improve model predictions of animal performance. Therefore, the purpose of this study was to (1) determine the NDF and NDIP degradation rates of four commonly fed corn (co)products using two in vitro methods and (2) compare the CPM-dairy model predictions with observed milk production from dairy cows fed diets containing 20 % corn distillers' grain.

MATERIALS AND METHODS

Product Description

Four corn (co)products were evaluated in this study. The first corn (co)product (Dakota Gold BPX DDGS; Poet Bio-Refinery of Dakota Gold Manufacturing, Sioux Falls, SD; **BPX-DDGS**) is a dried distillers grain plus solubles. The BPX-DDGS is derived from a low-heat processing method, which avoids heat prior to fermentation, reducing the incidence of heat-damage to proteins which are known to have lower digestibility in ruminants (Krishnamoorthy et al., 1982). The second corn (co)product (Dakota Gold HP DDG; Poet Bio-Refinery of Dakota Gold Manufacturing; **HP-DDG**), is a dried distillers grain derived from a novel processing method that physically

removes both bran (Dakota Bran; **BRAN**) and germ (Dakota Gold Corn Dehydrated Germ; **GERM**) prior to fermentation of the endosperm, resulting in a fourth (co)product that is high in protein. Solubles from this processing method are then added back to the BRAN and GERM. Feed samples were sent to Cumberland Valley Analytical Service (Hagerstown, MD) for chemical analysis (Table 2.1).

Table 2.1: Chemical analysis of four corn milling (co)products

Items	Feeds[1]			
	BPX-DDGS	HP-DDG	BRAN	GERM
DM, % as fed	91.5	92.4	90.9	91.4
TDN, % of DM	85.6	79.3	86.5	100.0
CP, % of DM	29.4	43.3	14.6	16.9
Soluble Protein, % of CP	17.2	3.4	34.0	39.7
ADF protein, % of DM	1.4	2.6	1.5	0.9
NDF protein, % of DM	2.8	4.0	1.8	3.2
NFC, % of DM	28.1	24.6	48.8	38.7
Starch, % of DM	6.5	9.7	31.1	25.4
Sugar, % of DM	3.2	1.6	5.3	10.4
Estimated digestibility, % of DM[2]				
RDP (kp = 0.04 h^{-1})	21.2	27.2	12.4	10.7
ADF, % of DM	9.8	11.4	6.8	6.4
NDF, % of DM	29.8	30.0	23.4	24.2
Lignin, % of DM	3.3	3.0	2.0	1.3
Fat, % of DM	10.4	3.9	9.3	17.3
Ash, % of DM	5.1	2.1	5.7	6.1

[1] Feeds analyzed: BPX-DDGS and HP-DDG = corn dried distillers grain (co)products where BPX-DDGS is derived from a low heat processing method and HP-DDG has high protein content, BRAN = corn bran with solubles, and GERM = dehydrated corn germ with solubles. Feeds were composites, homogenized from thirty separately processed batches.

[2] Computed using fractional rate of fermentation of the feed. The estimated fractional rate of passage (kp = 0.04 h^{-1}) is based on typical diets of dry cows, as predicted by the CPM-dairy Model (Boston et al., 2000).

Chemical and In Vitro Methods

Fiber residue. Thirty batch samples of four corn (co)products (n=120) were processed at the Ruminant Nutrition Laboratory at Texas A&M University. Each (co)product was hand ground using a mortar and pestle. Neutral detergent fiber residue (**NDR**) was obtained by using neutral detergent solution (FND20C; Ankom Technology, Macedon, NY) with heat-stable α-amylase (100 μL per 0.50 g of sample; Ankom Technology) but without sodium sulfite (Na_2SO_3) (Tedeschi et al., 2009).

In vitro anaerobic fermentation and gas production. In vitro anaerobic fermentation and gas production analysis was performed on the 120 samples of NDR. The apparatus used in the in vitro gas production (**IVGP**) technique to collect fermentation profiles of the (co)products is similar to that described in Pell and Schofield (1993). The technique requires an incubation chamber, to mimic rumen temperature (39°C), with a multi-plate stirrer, which houses fermentation flasks (125 mL Wheaton bottles). Flasks are attached to pressure sensors that connect to an analog to digital converter device that communicates with a computer software program that measures and records gas pressure at regular intervals for a predetermined period of time. Approximately 200 mg of ground NDR (BPX-DDGS, HP-DDG, BRAN and GERM) was weighed and transferred into respective 125 mL Wheaton bottles with Teflon covered stir bars, and dampened with 2.0 mL of ddH_2O, to prevent particle scattering during subsequent CO_2 flushing. Meanwhile, Goering and Van Soest's (1970) media was prepared, boiled and cooled to room temperature, while being flushed with CO_2. Resazurin was used in the media as an optical indicator of CO_2 saturation, turning from

purple to pink/colorless. Cysteine hydrochloride was then added, and media pH was measured to ensure optimal buffer range between 6.8 and 6.9. At this point 14 mL of media was transferred to fermentation bottles anaerobically, plugged with lightly greased butyl rubber stoppers, and crimp sealed. Bottles were then carefully placed inside the fermentation incubator and connected to their respective pressure sensors via needle insertion. Rumen fluid was collected from a non-lactating rumen cannulated Jersey cow grazing medium quality grass. Inoculum was filtered through 4 layers of cheesecloth and glass wool, into a flask continually flushed with CO_2 to prevent oxygen toxicity from altering existing microbial population. Once the fermentation chamber reached 39°C, 4 mL of rumen inoculum was injected with needle and syringe into each fermentation bottle. Pressure inside the bottles was equalized by piercing rubber stoppers with a needle for approximately 5 seconds, prior to recording. Once software recording was initialized, atmospheric pressure was recorded, and residual rumen inoculum pH was recorded. After 48 h of fermentation, bottles were depressurized, pH was measured, and 40 mL of neutral detergent solution (Van Soest et al., 1991) was added to each bottle which were then resealed and autoclaved for 60 min at 105ºC. Undegraded fiber was then filtered gravimetrically using Whatman 54 filter paper and oven dried.

Neutral detergent insoluble protein residue. Ten randomly selected sub-samples (30 g) were taken from each original BPX-DDGS, HP-DDG and GERM samples and combined to obtain new composite (co)products (300 g), for each feed respectively. The NDIP was measured following the procedures for in vitro true digestibility (Ankom Technology, Macedon, NY) and resulting fiber residue analyzed for nitrogen. Briefly,

rumen fluid was obtained from a ruminally cannulated British-breed steer fed medium quality hay. As described by Tedeschi et al. (2009), in vitro disappearance of the samples was measured using the Tilley and Terry (1963) method modified with the addition of 1 g per L of urea to the McDougall's buffer (Weiss, 1994). Seven time points were chosen to determine the rate of NDIP disappearance over time (**T0**, **T1**, **T3**, **T6**, **T12**, **T24**, and **T48** h). Feed samples were arranged in the Daisy II fermentation vessels (Ankom Technology, Macedon, NY), excluding T0, so that approximately 30 g (5 g per time point) of each feed were incubated per vessel. Media and rumen inoculum proportions were adjusted from the protocol so that substrate to solution ratio was 10 g per L. For each time point, fermented samples were removed from digestion jars, rinsed with distilled water and processed through the Ankom Fiber Analyzer (Ankom Technology, Macedon, NY). After NDR was dried and weighed, time points for respective feed samples were homogenized and ground. Protein content of the NDR was determined by combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation St. Joseph, MI).

Lactation Trial

Animals, experimental design, and treatments. Twenty-one primiparous and twenty-five multiparous Holstein cows averaging 22.9 ± 3.6 DIM and 1440.7 ± 126.3 kg BW were randomly assigned to one of four dietary treatments. Animals were blocked into groups of four according to parity and days fresh and randomly assigned to one of four dietary treatments. Animals began treatment when they were approximately 21

Table 2.2: Ingredient composition of the diets fed to lactating dairy cows formulated using the CPM-dairy model

	Diets[2]			
Diet ingredients, % of DM	A	B	C	D
Alfalfa Haylage	21.89	21.89	21.89	21.89
Corn Silage	20.7	20.7	20.7	20.7
BPX-DDGS[1]	19.9	9.95	4.98	0
HP-DDG[1]	0	9.95	14.93	19.9
Ground Corn	13.93	13.93	13.93	20.8
Alfalfa Hay	5.97	5.97	5.97	5.97
Soybean Hulls, ground	4.96	4.96	4.96	4.86
SBM	3.98	3.98	3.98	0
Soy Pass	2.89	2.89	2.89	0
Brome Hay	2.39	2.39	2.39	2.39
Limestone, ground	1.49	1.49	1.49	1.0
Sodium bicarbonate	0.9	0.9	0.9	0.9
Salt, NaCl	0.6	0.6	0.6	0.6
MagOx	0.18	0.18	0.18	0.3
Vit ADE	0.12	0.12	0.12	0.12
Trace mineral	0.1	0.1	0.1	0.1
Calcium diphosphate	0	0	0	0.5

[1]BPX-DDGS and HP-DDG are corn dried distillers grain (co)products, in which BPX-DDGS is derived from a low heat processing method and includes solubles, and HP-DDG is high in protein.
[2]Diet contained 20 % of corn (co)product, where A= 100 % BPX-DDGS, B= 50:50 mix of BPX-DDGS and HP-DDG, C= 25:75 mix of BPX-DDGS and HP-DDG, D= 100 % HP-DDG.

DIM and milk production was followed for the next 12 weeks; however, they were allowed 1 week for adaptation before beginning experimental measures. Dietary treatments were determined before animals began the experimental trial and remained on same dietary treatment throughout the experiment. Dietary treatments differed by type of corn milling (co)product and level of (co)product included in the TMR (Table 2.2). The first treatment contained 20% of the diet DM BPX-DDGS. The second treatment contained 20% HP-DDG. The third and fourth treatments contained 10% BPX-DDGS

and 10% HP-DDG, and 5% BPX-DDGS and 15% HP-DDG, respectively. Treatments were formulated using CPM-dairy model (Version 3.0.10) to meet or exceed nutritional requirements according to CPM-dairy model (Boston et al., 2000).

Animal care and use, milk collection, BW, BCS, and feed. All experimental procedures and animals were cared for according the guidelines by the University of Nebraska-Lincoln Institutional Care and Use Committee. Animals were housed in individual stalls at the University of Nebraska-Lincoln dairy research unit (Mead, NE) offered ad libitum water, and milked at 0730 and 1900. Weekly milk samples were collected during the AM and PM milking, preserved with 2-Bromo-2-nitropropane-1,3-diol and sent to Heart of America DHIA (Manhattan, KS) for laboratory analyses of fat, true protein, lactose, SNF, and MUN. Milk true protein, fat and lactose were analyzed for each sample using near-infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Milk component percentages were reported and yields were calculated based on MY and milk component percentage. Milk urea nitrogen concentration was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments).

Cows were fed individually at 0900 each day for ad libitum intake and approximately 10% refusal. The amount of feed offered was recorded daily and refusals were individually removed and weighed each day at 0800. Individual daily intake was calculated by subtracting refusal from amount fed. Corn silage and alfalfa haylage were monitered weekly for DM content using a microwave oven (Heinrichs and Ishler, 2000).

Table 2.3: Chemical composition of the diets fed to lactating dairy cows predicted by the CPM-dairy model

	Diets[1]			
Chemical, % of DM	A	B	C	D
DM, %	50.40	50.39	50.38	50.41
CP	19.34	20.68	21.35	19.09
RUP% CP	38.43	40.81	41.88	41.06
RDP%CP	61.57	59.19	58.12	58.94
Starch	18.46	18.46	18.46	23.43
NDF	33.79	34.29	35.54	34.08
ADF	21.7	22.05	22.22	22.12
Lignin	4.09	4.04	4.02	3.95
NE_L, Mcal/Kg	1.67	1.65	1.63	1.63
EE	4.80	4.08	3.72	3.5
NFC	35.55	34.84	34.49	37.29
Ash	9.84	9.54	9.39	9.01
Ca	1.02	1.05	1.06	0.94
Mg	0.35	0.38	0.39	0.38
P	0.41	0.46	0.49	0.38
K	1.48	1.57	1.61	1.24
S	0.34	0.33	0.33	0.25

[1]Diet contained 20 % of corn (co)product, where A= 100 % BPX-DDGS, B= 50:50 mix of BPX-DDGS and HP-DDG, C= 25:75 mix of BPX-DDGS and HP-DDG, D= 100 % HP-DDG.

Diet ingredient proportions were adjusted if DM content changed in the corn silage, alfalfa haylage, or both. Samples of total mixed rations were collected weekly and frozen for nutrient analysis (Table 2.3). Corn silage, alfalfa haylage, alfalfa hay, and brome hay were collected every three weeks and sent the same day to Cumberland Valley Analytical Services (Hagerstown, MD) for chemical analysis of DM (AOAC 2000; method 930.15), CP (AOAC 2000; method 990.03) ADICP (Leco FP-528 Nitrogen Combustion; St. Joseph, MI), ADF (AOAC 2000; method 973.18), NDF (Van Soest et al., 1991), lignin (Goering and Van Soest, 1970), crude fat (AOAC 2000; method

2003.05, ash (AOAC 2000; method 942.05) starch (Hall, 2009) , calculations of NFC , TDN, NEl, NEm, NEg (NRC, 2001), and minerals, Ca, P, Mg, K, S, Na, Fe, Mn, Zn, Cu, and Cl (AOAC 2000; method 985.01) . Particle size distribution of the rations was determined using the Penn State Particle Separator (Kononoff et al., 2003). Cows were individually weighed each week immediately after the AM milking. Body condition score was obtained each week using a 1 (extremely thin) to 5 (extremely fat) scale (Wildman et al., 1982) by a single trained individual.

Calculation of the Fractional Rate of Fermentation

Kinetic analysis of the 48 h gas production of the fermented NDR samples was performed similar to that described by Tedeschi et al. (2009) using the discrete exponential equation with lag time (Schofield et al., 1994) as shown in Eq. [1].

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

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 λ is lag time, h.

The gas production data for each feed were fitted to Eq. [1] using the GasFit System v. 3.5 (http://www.nutritionmodels.tamu/gasfit.html), which consists of a script that uses the port algorithm of the nls function of R (R Development Core Team, 2008) as described by Tedeschi et al. (2008a,b). The fractions rate of fermentation (**kf**, h^{-1}) was

obtained for each feed NDR and it was assumed to be the degradation rate of the available NDF.

The fractional rate of degradation of NDIP (kd, 1/h) was determined for each corn milling (co)product using a decay nonlinear equation as shown in Eq. [2]. The PROC NLIN of SAS (SAS Inst. Inc., Cary, NC) was used to converge the NDIP values.

$$NDIP = a \times \exp(-kd \times t) \quad [2]$$

where, a is the percent protein of the NDR and t is time.

CPM Model Simulations

To evaluate the potential impact of the newly obtained NDF and NDIP fractional rates of fermentation, previously determined in vitro described above, on ME and MP allowable milk, model simulations were performed for each animal using the CMP Dairy model (http://www.cpmdairy.com/Index.html; Boston et al., 2000; Tedeschi et al., 2008c). Model predictions, using each cow's biological information, observed milk production, and new rates were compared with predictions using the original feed dictionary rates for NDF and NDIP (7 and 0.5 %/h, respectively) for corn distillers' grain plus solubles. Simulations were evaluated as described by Tedeschi (2006) and assessment measurements (e.g. mean bias, r^2, accuracy – **Cb**) used to compare observed and model-predicted values.

Statistical Analysis

In vitro anaerobic fermentation and gas production. Statistical analysis was performed using PROC GLIMMIX of SAS (SAS Inst. Inc., Cary, NC), assuming an incomplete block design. The computerized IVGP chamber can house and record gas pressure of 22 Wheaton bottles simultaneously. Hence, several box runs were needed to ferment all 120 feed samples, in duplicate. Feeds were blocked by box run. Treatments were feeds BPX-DDGS, HP-DDG, BRAN and GERM, and were considered fixed effects, and blocks were assumed to be random.

Milk production and composition. The milk production and milk composition were analyzed using PROC MIXED of SAS (SAS Inst., Cary, NC). Cows were segregated by the time of their addition to the trial (Block) and lactation (Parity). Block, Parity, and cows within Block and Parity were assumed as random variables. Treatment and week of lactation, and their interaction, were analyzed as fixed effects. The interaction between treatment and week of lactation was removed if not significant at $P < 0.05$. Previous milk production (1 week) was used as a covariate. The interaction between the covariate and the fixed effect variables were included in the statistical model and removed if not significant at $P < 0.05$. The variance-(co)variance matrix structure for the random variables was Variance Components and for the repeated statement was auto-regressive order 1 for weeks of lactation. Orthogonal contrasts were used to evaluate linear, quadratic, and cubic patterns of milk production and milk composition.

RESULTS AND DISCUSSION

NDR Gas Production and Digestibility

Table 2.4 shows the fermentation characteristics and digestibility of the NDR and the fractional rate of degradation of the NDIP of the corn (co)products. Analysis of the gas production profiles revealed that the NDR of BRAN and GERM produced more total gas that HP-DDG and BPX-DDGS (14.75 and 14.62 vs. 12.69 and 8.94 mL, respectively; $P < 0.01$). Although Tedeschi et al. (2009) also reported that the NDR of BRAN produced the most total gas (40.1 mL), results of total gas production for BPX-DDGS, GERM and HP-DDG were different (33.7, 28.4 and 26.2 mL, respectively; $P < 0.0001$). Moreover, the NDR of the (co)products in Tedeschi et al. (2009) produced about twice as much total gas than the (co)products in the present study. However, the present study fermented more NDR feed samples than did Tedeschi et al. (2009). Additionally, fermentation profiles of the present study converged better when there was no adjustment for the gas production of the blanks used in the technique.

Fermentation rate was greatest for the NDR of BRAN (0.137 h^{-1}), followed by GERM (0.111 h^{-1}), and BPX-DDGS and HP-DDG (0.084 h^{-1} and 0.074 h^{-1}, respectively; $P < 0.01$). These results differed from those of Tedeschi et al. (2009) where HP-DDG had the most rapid fermentation rate (0.12 h^{-1}). However, Varga and Hoover (1983) reported more similar fermentation rates for the NDF of distillers grains (0.072 h^{-1}).

Lag time prior to fermentation was greatest for BRAN (0.577 h), and was zero h for BPX-DDGS, HP-DDG and GERM ($P < 0.01$). Tedeschi et al. (2009) also reported that lag time was greatest for BRAN (5.15 h), and that lag times of BPX-DDGS and HP-

DDG were not different from each other. However, Tedeschi et al. (2009) reported that GERM had the shortest lag time, unlike the results of this study, where GERM was not different from BPX-DDGS and HP-DDG.

Estimated NDR ruminal digestibility (Table 2.4) was calculated assuming passage rates (**kp**) of the CPM-dairy simulations for dry cows (0.04 h^{-1}), low- and high-producing (0.06 and 0.08 h^{-1}, respectively). Analysis showed that the NDR of BPX-DDGS would have the greatest ruminal digestibility, BRAN and GERM would be similar and have the lowest rumen degradable NDR, and the NDR of HP-DDG would have an intermediate digestibility.

Chemical analysis of the composite (co)products revealed that the NDIP content of BPX-DDGS and HP-DDG where the same (1.4 %, DM) and GERM had the greatest content (2.3 %, DM). Fractional rates of NDIP degradation for BPX-DDGS (0.07 h^{-1}) and HP-DDG (0.06 h^{-1}) were found to be similar to the rate used for corn distillers' grain plus solubles in the CPM-dairy feed dictionary (0.05 h^{-1}). The degradation rate for the NDIP of GERM was found to be much faster (0.64 h^{-1}) than the other (co)products. This is likely due to the difference in the type of proteins contained in the (co)products, specifically those found in the endosperm and germ components of the corn kernel. The endosperm contains mostly prolamins, which are deficient in several essential amino acids whereas the germ contains mostly glutelin, which have a more balanced amino acid profile than prolamines; both proteins have different solubilities (Shukla and Cheryan, 2001; Harvey and Oaks, 1974. Additionally, GERM,

Table 2.4: Comparison of the anaerobic fermentation dynamics of neutral detergent residues of four corn milling (co)products [1]

		Feeds[2]					
NDR[2]	n[1]	BPX-DDGS	HP-DDG	BRAN	GERM	SEM	P-value
Total gas production (mL per 100 g of DM)	198	8.94[c]	12.69[b]	14.75[a]	14.62[a]	0.52	< 0.01
Factional rate of fermentation (h^{-1})	198	0.084[c]	0.074[c]	0.137[a]	0.111[b]	0.01	< 0.01
Lag time (h)	198	0.00[b]	0.00[b]	0.58[a]	0.00[b]	0.38	< 0.01
Estimated NDR ruminal digestibility[3]							
Fractional kp = 0.04 h^{-1}	198	27.8[a]	23.8[b]	20.8[c]	20.2[c]	0.55	< 0.01
Fractional kp = 0.06 h^{-1}	198	24.8[a]	21.0[b]	19.1[c]	18.3[c]	0.56	< 0.01
Fractional kp = 0.08 h^{-1}	198	22.6[a]	19.0[b]	17.7[c]	16.8[c]	0.55	< 0.01
Fractional rate of NDIP degradation, h^{-1}	63	0.07[b]	0.06[b]	--	0.64[a]	0.02	< 0.01

[a-c] Within a row, LSM without a common superscript letter differ P-value <0.0001

[1]Values are Least squares means (LSM) and SEM is the average of the SE of the LSM. Number of profiles that converged out of 240 observations

[2]BPX-DDGS and HP-DDG are corn dried distillers grain (co)products, in which BPX-DDGS is derived from a low heat processing method and includes solubles, and HP-DDG is high in protein, BRAN = corn bran, GERM = corn germ dehydrated. NDR= neutral detergent residue with α-amylase but without sodium sulfite.

[3]Computed using fraction rate of fermentation of the neutral detergent residue. Estimated fractional passage rates (kp : 0.04,0.06, and 0.08 h^{-1}) were based on typical diets of dry cows , low- and high- lactating cows as predicted by the CPM-dairy (Boston et al., 2000).

in its intact form, was reported to have the greatest digestibility of the four (co)products (Tedeschi et al., 2009). The amino acid profile and bioavailability of other nutrients to microbes likely contributed to the more rapid rate of NDIP degradation. However, GERM was not included in the lactation trial diets and was not further analyzed, but could serve as a reference value for any future research on this (co)product.

Milk Production

Results of the milk production analysis revealed that week affected fat corrected milk (**FCM**), milk fat percentage and yield and milk protein percentage and yield. Previous milk production (covariate) affected FCM, milk protein % and mild fat yield. There was an interaction of week and covariate for FCM, milk protein and fat yields. Results of the diet effects on milk production, shown in Table 2.5, indicated FCM tended to decrease linearly as percent of BPX-DDGS decreased in the diet ($P = 0.08$). This may have been due to the amount of fat in each ration which decreased as BPX-DDGS decreased in the diet from A to D, (4.8, 4.08, 3.72 and 3.5 % of diet DM, respectively). Interestingly, percent milk fat was different among diets showing a cubic effect ($P = 0.03$). Fat yield also tended to have a cubic effect ($P = 0.09$). Percentage of milk protein was not affected by diet, however milk protein yield tended to decrease linearly as percent of BPX-DDGS decreased in the diet ($P = 0.06$). This trend is similar to that in Janicek et al. (2008) where increasing percentages of a DDGS, chemically similar to BPX-DDGS, in the diet increased milk protein yield linearly ($P = 0.03$). This may have

been a result of increased energy availability for milk protein synthesis and greater milk yield (Janicek et al., 2008).

Model Performance

Model predicted (Table 2.6) values were not different between our newly measured degradation rates and the original feed library values for corn distillers' grain with solubles. This was likely because rates determined in vitro were relatively similar to the original values of the feed dictionary. Additionally, effects of the evaluation were even more obscured since the diets only contained 20 % of the (co)products.

Resistant coefficient of determination indicated large deviation of observed milk production from the best-fit regression (closer to 1 is better), but was greatest for diet A and lowest for Diet B. Mean bias (closer to 0 is better) was largest for individual diet A and smallest when all diets were considered. Model accuracy (closer to 1 is better) was evaluated to be less than 50% accurate among diets but greatest for diet D and lowest for diet B.

Based on the (co)products used in this study, our findings suggested that there was a tendency to decrease milk production when the percentage of BPX-DDGS was decreased in the diet compared to HP-DDG. Predictions of the calibrated CPM-dairy model using the NDF and NDIP degradation rates determined in vitro where not different from predictions using current feed dictionary values for corn distillers' grain plus solubles. Model accuracy was low when individual cow performance was simulated. However, accuracy and mean bias were best when all animals were

Table 2.5: Effect of feeding two corn milling (co)products on milk production

Item		Diets[2]					P-value		
	n	A	B	C	D	SEM	Linear	Quad	Cubic
3.5 % FCM[1], kg/d	44	38.71	35.19	36.45	33.86	2.61	0.08	0.89	0.36
Fat, %	44	3.87[a]	3.55[b]	3.86[a]	3.67[ab]	0.11	0.34	0.38	0.03
Fat yield, kg/d	44	1.53	1.32	1.41	1.29	0.11	0.26	0.31	0.09
Protein, %	44	2.89	2.88	2.91	2.88	0.04	0.89	0.92	0.56
Protein yield, kg/d	44	1.16	1.06	1.07	1.03	0.06	0.06	0.34	0.19

[1]3.5 % FCM = 0.432 × milk (kg/d) + 16.23 × fat (kg/d) (Tyrrell and Reid, 1965).
[2]Diets contained 20 % of corn (co)product, where A= 100 % BPX-DDGS, B= 50:50 mix of BPX-DDGS and HP-DDG, C= 25:75 mix of BPX-DDGS and HP-DDG, D= 100 % HP-DDG.

Table 2.6: Model accuracy of diet predictions of the CPM-dairy model

	Measurements[1]						
	r^2		Mean Bias		Model accuracy (Cb)		
Diet[2]	Original	Calibrated	Original	Calibrated	Original	Calibrated	*P*-value
A	0.24	0.23	7.53	7.31	0.36	0.37	0.49
B	0.05	0.05	-4.43	-4.59	0.273	0.28	0.50
C	0.18	0.17	4.46	4.33	0.41	0.41	0.50
D	0.20	0.20	-4.66	-4.77	0.47	0.47	0.50
All	0.03	0.03	1.16	0.10	0.45	0.46	0.49

[1]Measurments were r^2= resistant coefficient of determination (closer to 1 is better), Mean bias (closer to 0 is better, Cb= correction bias (model accuracy) (closer to 1 is better).
[2]Diet contained 20 % of corn (co)product, where A= 100 % BPX-DDGS, B= 50:50 mix of BPX-DDGS and HP-DDG, C= 25:75 mix of BPX-DDGS and HP-DDG, D= 100 % HP-DDG.

considered. This suggests the CPM-dairy model is calibrated to better predict the performance of a large group of animals rather than individuals or small groups as discussed by Tedeschi et al. (2008c).

CHAPTER III

EVALUATION OF IN VITRO GAS PRODUCTION AND RUMEN BACTERIAL POPULATIONS FERMENTING CORN MILLING (CO)PRODUCTS *

INTRODUCTION

The corn-ethanol dry milling industry produces several corn (co)products that can be utilized in ruminant feed rations. Use of these products is sometimes limited because of variation in nutrient composition across dried distillers grains with (**DDGS**) or without (**DDG**) solubles, products of different manufacturers (Spiehs et al., 2002) as well as within a single ethanol plant (Belyea et al., 2004). As more information about the quality of corn (co)products becomes available, new strategies of (co)product feeding will be developed. Feedstuff processing methods have been shown to affect feed efficiency, production (Anderson et al., 2006), and quality (Powers et al., 1995; Tedeschi et al., 2009). Tedeschi et al. (2009) reported that the proportion of fiber digested by rumen microbes in vitro was affected not only by the degree of feed processing but also by fat removal. The detrimental effects on milk quality of inclusion of unsaturated fat in dairy cow rations (Macleod and Wood, 1972) has led to a proposed method that involves deoiling or defatting (co)products. Exploring the chemical composition and fermentation dynamics of these (co)products of different processing methods, could yield more

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accurate and effective feeding strategies.

The in vitro gas production (**IVGP**) technique, as described by Tedeschi et al. (2009), is a valuable tool that describes the fermentability of ruminant feeds. It could be advantageous to link rumen bacterial population shifts with these fermentation results, which may increase our knowledge of corn (co)product fermentation dynamics, end-products, and the most efficacious processing method. One of the newest methods to identify bacteria utilizes DNA pyrosequencing, which characterizes bacterial populations on a phylogenetic basis (Dowd et al., 2008a,b,c). The present study uses the 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (**bTEFAP**) technique. This method is rapidly evolving to improve precision in genotyping (Armougom and Raoult, 2009). Understanding the broader effects of feed processing and bacterial population shifts in response to nutrients contained in different corn (co)products may be valuable to improve production efficiency.

Tedeschi et al. (2009) previously published that defatting corn (co)products increased total gas production, reduced lag time, and reduced rate of fermentation. Our hypothesis is that the defatting process would increase fermentability of corn (co)products and alter bacterial populations. Therefore, the objective of this study was to evaluate the fermentation dynamics, using the IVGP technique, of 2 commonly fed corn (co)products in their intact and defatted forms, and to investigate the shifts of the predominant rumen bacterial populations using the novel molecular technique bTEFAP.

MATERIALS AND METHODS

Sample Description and Chemical Analysis

Two corn (co)products were used in this study. The first corn (co)product (Dakota Gold BPX DDGS; Poet Bio-Refinery of Dakota Gold Manufacturing, Sioux Falls, SD; **BPX**) is a DDGS resulting from a low-heat processing method before fermentation, which is assumed to have less heat-damaged protein. The second corn (co)product (Dakota Gold HP DDG; Poet Bio-Refinery of Dakota Gold Manufacturing; **HP**), is a value-added corn (co)product using a patented process that physically removes both bran and germ before endosperm fermentation, resulting in a high-protein (co)product. Additionally, the corn condensed distillers solubles are not added back to HP, unlike in the BPX product. Thirty samples of HP and BPX, respectively, were acquired, each sample representing a separate batch. Ten samples of each feed were randomly chosen and 1 g of each was combined to produce a new composite sample for BPX and HP, respectively. These composites were utilized as either intact or defatted before their in vitro incubation. Alfalfa hay was used as an internal laboratory standard. All chemical analyses were performed by Cumberland Valley Analytical Services (Hagerstown, MD). Analyses of the composite samples are shown in Table 3.1. The chemical analysis revealed the differing nutrient compositions of BPX and HP, especially in protein and fat content.

Defatted Residue

Defatted corn (co)product residues were obtained using the AOAC (2000; method 971.09). Extraction was performed using a 1,000-mL Soxhlet extractor and

Table 3.1: Chemical analysis of three intact feeds commonly fed in dairy cow rations

Items	Feeds[1]		
	Alfalfa Hay	BPX	HP
DM, % as fed	92.6	91.7	92.9
CP, % DM	21.2	28.2	42.4
Soluble protein, % CP	37.3	4.21	3.13
ADF protein, % DM	1.30	1.44	2.65
NDF protein, % DM	4.60	4.72	8.50
Estimated ruminal digestibility, % DM[2]			
RDP (kp = 0.04 h^{-1})	14.5	17.9	23.3
RUP (kp = 0.04 h^{-1})	6.70	10.3	19.1
NFC, % DM	26.3	27.6	22.9
Starch, % DM	1.10	5.77	8.38
Sugar, % DM	4.50	4.20	2.30
ADF, % DM	36.3	7.92	10.1
NDF, % DM	44.3	32.0	36.5
Lignin, % DM	7.70	1.80	2.07
Fat, % DM	2.00	11.4	3.88
Ash, % DM	2.00	5.51	2.84

[1]Feeds analyzed, Alfalfa hay used as an internal laboratory standard feed, BPX and HP-DDG = corn dried distillers grain (co)products where BPX undergoes a low heat process prior to kernel separation and HP has high protein content.
[2]Computed using fractional rate of fermentation of the intact feed. The estimated fractional rate of passage (kp = 0.04 h^{-1}) is based on typical diets of dry cows, as predicted by the CPM-dairy Model (Boston et al., 2000).

Friedrichs condenser. Whole samples (2 g) of HP and BPX were tightly wrapped in Whatman #54 paper, inserted into a thimble, and extracted with petroleum ether at condensation rate of 2 to 4 drops per second for 1 h. Samples were removed and dried at 60°C overnight.

In Vitro Anaerobic Fermentation and Gas Production

The in vitro anaerobic fermentation chamber as described by Tedeschi et al. (2009) was used to obtain the gas production pattern resulting from the fermentation of the intact or defatted composites. Briefly, the instrument consisted of an incubator with multi-plate stirrers, pressure sensors connected to 125-mL Wheaton incubation bottles, an analog to digital converter device, and a PC-compatible computer with Pico Technology software (Pico Technology, Eaton Socon, Cambridgeshire, UK). Composite samples (0.20 g) were transferred into 125-mL Wheaton bottles and then dampened with 2.0 mL of distilled water to prevent particle scattering. Bottles were then flushed with CO_2 to create an in vitro anaerobic atmosphere. Goering and Van Soest (1970) media (14 mL) was transferred to each bottle using strict anaerobic technique. Bottles were closed with lightly greased butyl rubber stoppers, crimp sealed, placed in the fermentation chamber, and inserted with respective sensor needles. Rumen inoculum was collected from a nonlactating, rumen-cannulated Jersey cow, with free access to medium to low quality grass and hay with salt and balanced mineral supplementation. The rumen fluid was filtered through 1 layer of cheesecloth and then again through glass wool, with continuous flushing of CO_2. Once the internal temperature of the

fermentation chamber reached 39°C, 4 mL of the filtered rumen fluid inoculum was injected into each bottle. After inoculation, the pressure was removed from the bottles by inserting needles into the stoppers for approximately 5 s. Once all bottles were equilibrated, the fermentation chamber was closed and the software began recording the accumulating gas pressure. The pressure was recorded every 5 min for 24 or 48 h. After 24 h, selected samples were quickly removed from the chamber. The remaining duplicate samples were removed at 48 h and final pH was recorded. Fermented samples were transferred into (50-mL) plastic BD Falcon conical tubes (BD Biosciences, San Jose, CA) for transporting purposes. Tubes were then immediately set in ice water to stop fermentation, and frozen overnight.

Pyrosequencing Analysis

The bTEFAP technique was used as described by Dowd et al. (2008a,b,c) to assess bacterial population. Frozen samples were shipped overnight on dry ice to the Research and Testing Laboratory (Lubbock, TX) for bTEFAP analyses as described previously (Dowd et al., 2008a,b,c). This new bTEFAP approach is based upon the same principles but utilizes titanium reagents and titanium procedures (Roche Diagnostics, Indianapolis, IN) and a 1-step PCR rather than a 2-step labeling reaction, a mixture of Hot Start and HotStar high fidelity Taq polymerases (Qiagen, Valencia, CA), and amplicons originating from the 27F region numbered in relation to *Escherichia coli* rRNA . Briefly, genomic DNA was extracted from fermented samples using a QIAmp DNA mini kit, concentrations equalized and DNA prepared for bTEFAP as described

previously (Dowd et al., 2008c; Wolcott et al., 2009a,b). All postsequencing processing was completed using custom-written software at the Research and Testing Laboratory (Lubbock, TX) as described previously (Dowd et al., 2008c; Wolcott et al., 2009a,b). Sequences generated (Research and Testing Laboratory) as part of the bTEFAP methodology were trimmed based upon Q20 quality criteria. Tags incorporated into each sequence as part of the bTEFAP process were utilized to individually identify sequences derived from each sample. Tags that did not have 100% homology to the original sample tag designation were not considered. The sequences from each sample were then separately compiled and tags removed from the sequences. Following removal of tags, sequences <200 bp were depleted with the final dataset averaging 400 bp with a range of 200 to 520 bp. Sequences were processed to remove chimeras using B2C2 software, which is described and freely available from Research and Testing Laboratory (http://www.researchandtesting.com/B2C2.html). The resulting data averaged 2,200 sequences per sample. Taxonomic designations were assigned using BLASTn against 16S database derived and continually updated from GenBank (http://ncbi. nlm.nih.gov). Best-hits were utilized along with secondary postprocessing algorithms to obtain taxonomic information as has been described previously (Dowd et al., 2008a; Acosta-Martinez et al., 2009). Phylogenetic assignments were based upon National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) taxonomic designations.

Statistical Analyses

Statistical analyses were done with SAS v. 9.2 (SAS Inst. Inc., Cary, NC) and R v. 2.9 (R Development Core Team, 2009).

Gas production data. The kinetic analysis of the 48-h cumulative gas production was evaluated using several nonlinear functions (Schofield et al., 1994). The nonlinear function with the lowest sum of square errors was selected. The nonlinear fitting was performed using GasFit (http://nutritionmodels.tamu.edu/gasfit.htm). GasFit executes specific R scripts to perform the convergence of gas production data, using the nls function (Bates and Chambers, 1993) and the "port" algorithm (Fox et al., 1978; Gay, 1990). Preliminary results indicated the exponential with discrete lag (Eq. [1]) and the logistic 2-pool (Eq. [2]) nonlinear functions had the lowest sum of square of errors; therefore, parameters of these nonlinear functions were compared using the statistical models described in Eqs. [3] to [5]:

$$Y = \begin{cases} a \times (1 - \exp(-b \times (t - c))); \forall t \geq c \\ 0; \forall t < c \end{cases} \quad [1]$$

$$Y = \frac{a}{1 + \exp(2 + 4 \times b \times (c - t))} + \frac{d}{1 + \exp(2 + 4 \times e \times (c - t))} \quad [2]$$

where a represents the asymptote, mL; b represents the fractional degradation rate, h-1; c represents lag time, h; d represents the asymptote of the second pool (assumed to be fiber), mL; and e represents the fractional degradation rate of the second pool, h-1.

Pyrosequencing data and pH measures. The pH data from the IVGP and data from the pyrosequencing analysis was analyzed as completely randomized design (CRD) with factorial arrangements using the PROC MIXED (Kuehl, 2000; Littell et al., 2006).

No data transformation was used because preliminary analysis indicated no improvement in normality of the residue. Least-square means were used for multiple comparisons using the Tukey's adjustment for the P-value. A three-way factorial arrangement of 2 feeds (HP or BPX) × 2 forms (intact or defatted) × 2 incubation time (24 or 48 h) was used (n = 16). The statistical model is shown in Eq. [3]. Only data applicable to all three blocks were utilized to analyze significant differences in pH, between the two feeds in question.

$$Yijkl = \mu + Fd_i + Fm_j + T_k + Fd\times Fm_{ij} + Fd\times T_{ik} + Fm\times T_{jk} + Fd\times Fm\times T_{ijk} + R_{l(ijk)} + e_{ijkl} \quad [3]$$

where Y was the measured variable, Fd was HP or BPX, Fm was intact of defatted feeds, T was incubation time (24 and 48 h), R was a random effect of replicate within feeds, form, and incubation time, and e was the random error.

When the alfalfa hay data was used, only intact form data was used because alfalfa hay did not have a defatted form. In this case, a two-way factorial arrangement of 3 feeds (alfalfa hay, HP, and BPX) × 2 incubation times (24 and 48 h) was used. The statistical model is shown in Eq. [4].

$$Yijk = \mu + Fdi + Tj + Fd\times Tij + Rk(ij) + eijk \quad [4]$$

where Y was the measured variable, Fd was alfalfa hay, HP, and BPX, T was incubation time (24 and 48 h), R was a random effect of replicate within feeds and incubation time, and e was the random error.

Table 3.2: Prominent bacterial species identified by 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing grouped by their affinitive substrate guild

Species	Substrate guild [1]									
	C	H	St	Pec	Su	Pro	Li	FC	NFC	La
Anaerovibrio lipolyticus							Li			
Bacteroides sp			St						NFC	
Butyrivibrio sp	C	H		Pec		Pro		FC	NFC	
Clostridium aminophilum						Pro				
Eubacterium ruminantium			St		Su				NFC	
Fibrobacter sp	C							FC		
Lachnospira sp				Pec						
Lactococcus lactis					Su				NFC	
Lactococcus sp					Su				NFC	
Megasphaera elsdenii						Pro				La
Prevotella bryantii		H		Pec				FC	NFC	
Prevotella sp		H	St	Pec		Pro		FC	NFC	
Ruminococcus sp	C	H						FC		
Selenomonas sp					Su				NFC	La
Streptococcus sp			St	Pec	Su				NFC	
Succinimonas sp			St						NFC	
Succinivibrio dextrinosolvens				Pec					NFC	
Treponema bryantii				Pec	Su		Li		NFC	

[1]C = Cellulose, H = Hemicellulose, St = Starch, Pec = Pectin, Su = Sugar, Pro = Protein, Li = Lipid, La = Lactate.

Bacterial populations were identified by their partial DNA at the species level and were reported as a percentage of the total DNA of all species identified. Because of the large number of species identified, with many found at small percentages, only substantiated bacteria or highly prevalent species (accounting for >5% of all species in at least one in vitro fermentation bottle) were used in our analyses. Species were then grouped by their known substrate affinities for cellulose, hemicellulose, pectin, starch, sugars, protein, lipid, and lactate according to Dehority (2003), Russell (2002), and Church (1988) (Table 3.2). Because of this type of grouping, bacterial species may belong to one or more guilds. The bacterial species used for the purpose of this research and their substrate guilds are shown in Table 3.2.

RESULTS AND DISCUSSION

Gas Production Analysis

Table 3.3 shows that there was no effect of feed (**Fd**), form (**Fm**), or their interaction on the parameters of the logistic 2-pool nonlinear function. There was also no effect on total gas production for the exponential with discrete lag nonlinear function. However, for the exponential with discrete lag nonlinear function, 2 interactions were observed in which intact corn (co)products had faster fractional degradation rates than defatted corn (co)products (0.20 vs. 0.12 h^{-1}, respectively; $P = 0.03$) and defatted corn (co)products had shorter lag times than their intact forms (1.99 vs. 1.24 h^{-1}; $P < 0.01$). Lag times of intact corn (co)products were not different (2.03 vs. 1.94 h^{-1}, respectively; $P = 0.51$) but defatting BPX decreased the lag time compared with defatting HP (0.81

vs. 1.66 h^{-1}, respectively; $P < 0.01$). These findings are somewhat dissimilar to that reported by Tedeschi et al. (2009) in which intact HP and BPX had faster fractional rates of degradation and longer lag times than their defatted forms. This may be due to variation in feed composition between the current and previous studies. Even though the total gas production was similar among feeds, intact BPX fermented faster than defatted BPX and alfalfa hay (0.22, 0.10, and 0.11 h^{-1}, respectively; $P = 0.018$). Although the fractional fermentation rate observed in this study was greater than that reported by Tedeschi et al. (2009), the ratio of the fractional fermentation rate of the intact to defatted BPX was similar (2.20 vs. 2.70 h^{-1}), suggesting that intact BPX was digested more quickly than its defatted form. However, defatted BPX was fermented sooner than the intact form (0.81 vs. 2.03 h^{-1}, respectively; $P = 0.02$). The increased lag time of the intact feed is likely due to free fatty acids inhibiting the growth of microflora. Maczulak et al. (1981) reported detrimental effects of several long-chain fatty acids on the growth of 7 fiber-degrading rumen bacteria. Jenkins (1993) also suggested that substrates could be subject to lipid coating along with the bacterial hydrolytic enzymes. Thus, bacteria degrading defatted feeds are uninhibited. Additionally, the relative increase of fiber upon fat removal may contribute to the defatted (co)product's slower fermentation rate. Therefore, based on our results, defatting BPX decreased its lag time and fractional fermentation rate by at least half ($P < 0.02$).

Table 3.3: Comparison of the dynamics of the in vitro fermentation of alfalfa hay and two intact and defatted corn milling (co)products using two nonlinear functions

Items[1]	Alfalfa	BPX		HP		SEM	*P*-value
	Hay	Intact	Defatted	Intact	Defatted		
Logistic 2-pools							
a, mL	11.0	6.59	11.3	13.5	13.1	3.42	0.38
b, h^{-1}	0.13	0.09	0.16	0.15	0.15	0.05	0.60
c, h	2.92	2.44	2.87	2.60	2.65	0.42	0.77
d, mL	5.97	7.79	9.39	4.36	5.15	2.75	0.45
e, h^{-1}	0.03	0.09	0.02	0.02	0.02	0.04	0.52
Exponential							
Total gas, mL	16.4	13.1	18.3	16.2	17.0	2.63	0.47
Fermentation rate, h^{-1}	0.11^{a}	0.22^{b}	0.10^{a}	0.18^{ab}	0.15^{ab}	0.02	0.02
Lag time, h	1.66^{ab}	2.03^{b}	0.81^{a}	1.94^{b}	1.66^{ab}	0.22	0.02

[a,b,c] Within a row, LSM without a common superscript letter differ ($P < 0.05$).

[1] a represents the asymptote, mL; b represents the fractional degradation rate, h^{-1}; c represents lag time, h; d represents the asymptote of the second pool (assumed to be fiber), mL; and e represents the fractional degradation rate of the second pool, h^{-1}.

Table 3.4: Effects of feed, time and form, and their interactions on pH and % of bacterial DNA recovered from mixed ruminal fluid fermented in vitro

Items	Feed		Time, h		Form		SEM	*P*-values				
	BPX	HP	24	48	I	D		Feed	Time	Form	Fd×Fm[4]	T×Fm[4]
pH	6.52	6.51	6.56[a]	6.46[b]	6.54	6.48	0.02	0.65	<0.01	0.06	0.15	0.72
Guild[3]												
FC, %	29.3[b]	36.2[a]	37.2[a]	28.3[b]	26.8[b]	38.7[a]	1.16	<0.01	<0.01	<0.01	0.05	0.04
Cellulose, %	5.99	5.12	5.09	6.03	5.47	5.65	0.60	0.34	0.30	0.83	<0.01	0.96
Hemi, %	28.5[b]	36.2[a]	36.8[a]	28.0[b]	26.8[b]	37.9[a]	1.16	<0.01	<0.01	<0.01	0.10	0.04
NFC, %	46.1	47.1	50.5[a]	42.8[b]	47.8	45.5	1.80	0.70	0.02	0.40	0.25	0.02
Starch, %	34.9	39.1	40.0[a]	34.0[b]	35.9	38.1	1.24	0.04	0.01	0.26	0.12	<0.01
Pectin, %	39.3[b]	43.7[a]	45.9[a]	37.1[b]	41.6	41.4	1.10	0.02	<0.01	0.89	0.62	<0.01
Sugar, %	16.7[a]	10.1[b]	11.4	15.4	19.9[a]	6.87[b]	1.75	0.03	0.14	<0.01	0.04	0.12
Protein, %	28.1[b]	35.2[a]	36.4[a]	26.9[b]	26.1[b]	37.2[a]	0.92	<0.01	<0.01	<0.01	0.19	0.06
Fat, %	2.14	1.62	1.26[b]	2.49[a]	1.15[b]	2.60[a]	0.19	0.09	<0.01	<0.01	0.80	0.02
Lactate, %	2.77	1.73	3.21[a]	1.23[b]	3.06[a]	1.44[b]	0.37	0.08	<0.01	0.02	0.11	0.72

[a-b] Within a row, LSM without a common superscript letter differ ($P < 0.05$).

[1]Values are least squares means (LSM) and SEM is the average of the SE of the LSM.

[2]BPX and HP = corn dried distillers grain (co)products where BPX undergoes a low heat process and HP has high protein content, I = Intact Feed, D = Defatted Feed, Hemi= Hemicellulose.

[3]Guild = bacteria combined into a substrate-utilizing group; given as % of all bacterial DNA recovered from each fermented sample.

[4]Fd|Fm = interaction of Feed and Form, T×Fm = interaction of Time and Form.

Table 3.5: Effects of the interaction of feed and time on the % of bacterial DNA recovered from mixed ruminal fluid fermented in vitro

Guild[3]		Feeds[2]							
		Alfalfa Hay		BPX		HP			
	n	24 (h)	48 (h)	24 (h)	48 (h)	24 (h)	48 (h)	SEM	*P*-value
FC, %	12	43.8^{a}	20.4^{c}	22.9^{c}	20.0^{c}	35.5^{ab}	28.7^{bc}	1.96	< 0.01
Cellulose, %[4]	12	8.36	11.4	2.88	5.05	7.06	6.88	1.60	0.60
Hemicellulose, %	12	42.2^{a}	20.0^{c}	22.9^{c}	20.0^{c}	35.5^{ab}	28.7^{bc}	1.87	< 0.01
NFC, %	12	47.5^{a}	20.4^{b}	47.8^{a}	49.8^{a}	47.5^{a}	45.9^{a}	3.66	0.01
Starch, %	12	36.7^{a}	11.2^{b}	37.3^{a}	33.3^{a}	33.9^{a}	39.2^{a}	1.55	< 0.01
Pectin, %	12	45.7^{a}	17.5^{b}	42.0^{a}	37.6^{a}	43.5^{a}	43.4^{a}	1.77	< 0.01
Sugar, %[4]	12	2.84	3.29	22.9	29.6	8.59	18.6	3.91	0.50
Protein, %	12	41.5^{a}	17.0^{c}	22.8^{c}	19.7^{c}	35.1^{ab}	26.7^{bc}	1.87	< 0.01
Fat, %[4]	12	0.33	1.73	1.26	1.49	0.63	1.22	0.44	0.45
Lactate, %	12	1.13^{b}	0.96^{b}	5.38^{a}	2.71^{b}	2.85^{b}	1.29^{b}	0.38	0.05

[a-c]Within a row, LSM without a common superscript letter differ ($P < 0.05$).

[1]Values are least squares means (LSM) and SEM is the average of the SE of the LSM.

[2]Computed using intact feeds where HAY = Alfalfa hay, an internal laboratory feed standard, BPX and HP-DDG = corn dried distillers grain (co)products where BPX is undergoes a low heat process and HP has high protein content.

[3]Guild = Bacteria combined into a substrate-utilizing group; given as % of all bacterial DNA recovered from each fermented sample.

[4]Main effects were different; see text.

pH Effects

Table 3.4 compares HP and BPX and demonstrates the effect of time (**T**), Fm, and their interactions on pH. No difference in the average pH between BPX and HP was observed, nor was there a difference between forms, a likely result of the buffering media in the fermentation mixture. Time had an effect on pH ($P < 0.01$), showing a decrease in the average pH from 6.56 at 24 h to 6.46 at 48 h. However, research has shown that variations of pH between 7.0 and 6.2 have only minor influence on microbial activity (Shriver et al., 1986; Slyter, 1986). This decrease in pH is likely due to the production of VFA from fermentation and lack of end-product removal in the in vitro technique. Thus, it does not appear that pH had an effect on the bacterial shifts of this experiment.

Pyrosequencing Analysis

Analysis of intact feeds and time effects. Table 3.5 compares the effects that the interaction of the intact feeds (alfalfa hay, BPX, and HP) and time (24 and 48 h) had on bacterial populations recovered from the mixed ruminal fluid fermented in vitro. Interactions of Fd and T were seen, with the exception of the cellulolytic, sugar-, and fat utilizing guilds. The fiber carbohydrate (**FC**)-utilizing bacteria populations decreased over time for alfalfa hay (43.8 vs. 20.4%), whereas they did not change for BPX or HP. This may have been due to the inhibitory effects of long-chain fatty acids contained in the (co)products on the fibrolytic bacteria and their digestive enzymes. This is also supported by the greater population variance in the HP (35.5 vs. 28.7%) relative to the

BPX (22.9 vs. 20.0%) over time, when considering that HP contained much less ether extract than BPX.

For the NFC-utilizing bacterial guild, the Fd × T interaction revealed that populations decreased over time (47.5 vs. 20.4%) for alfalfa hay, whereas they did not change for the (co)products. It is unclear why this occurred but we speculate a difference in nutrient composition of the NFC fraction.

The Fd × T interaction of the proteolytic guild showed that populations degrading the alfalfa hay decreased over time (41.5 vs. 17.0%), whereas there was no difference for BPX or HP over time. However, populations in HP at 24 h were greater compared with those in BPX at 24 and 48 h. This was expected because HP was the high protein feed. The large percentage of bacteria degrading the alfalfa hay is likely attributed to the large percentage of soluble protein contained in the alfalfa hay compared with the amounts in the (co)products (37.3 vs. 4.21 and 3.13%). This may also explain the significant decrease in the bacterial population from 24 to 48 h, because that soluble protein substrate would have diminished more quickly than the other less-soluble protein fractions contained in the (co)products.

The Fd × T interaction for the lactate-utilizing guild showed a decrease in the bacterial population over time for BPX, whereas there was no difference for alfalfa hay or HP over time. The BPX at 24 h had the greatest population (5.38%) of lactate utilizing bacteria. This may be explained by the main effects of the sugar guild, which indicated that BPX supported a much larger population of sugar-utilizing bacteria than alfalfa hay and HP, allowing more cross-feeding of these 2 bacterial guilds.

Analysis of feed, time, and form effects. The main effects of the 2 feeds (BPX and HP), over time (24 and 48 h) and form (intact and defatted) and their interactions are shown in Table 3.4. As expected, the main effects show that HP supported a greater percentage of FC-utilizing bacteria than did BPX, likely because of the greater percentage of NDF contained in HP (36.5 vs. 32.0%). A T × Fm interaction was observed for the FC guild, where bacterial populations degrading the intact feeds were not different over time (29.2 vs. 24.4%), whereas bacterial populations degrading the defatted feeds decreased over time (44.3 vs. 31.6%). The defatted forms supported a greater percentage of the FC-utilizing bacteria probably due to lack of interference by lipid coating and the relative increase of substrate upon fat removal. The smaller population degrading the intact feeds was likely due to the inhibitory effect of long-chain fatty acid release from the intact feeds over time. The NFC-, starch-, and pectin-utilizing guilds decreased over time. The T × Fm interaction revealed that the bacterial populations degrading the defatted forms decreased over time for NFC (53.3 vs. 37.7%; $P = 0.02$), starch (44.3 vs. 31.8%; $P < 0.01$), and pectin (49.1 vs. 33.7%; $P < 0.01$), whereas the intact forms did not change over time (47.7 vs. 47.8%, 35.6 vs. 36.2%, and 42.7 vs. 40.5%, respectively). Pectinolytic bacteria were affected by feed. Although pectin was not measured in the chemical analysis, it is known that its solubility characteristics overlap with that of hemicellulose, causing an overestimation of the hemicellulose fraction when using the detergent system (Van Soest, 1994). Although other smaller fractions are lost, we can estimate that hemicellulose and pectin account for most of the difference between NDF and ADF. By calculating the difference between

the NDF and ADF content of BPX and HP, we can estimate the hemicellulose component (8 vs. 25.1%, respectively). This is a likely reason that pectinolytic and hemicellulolytic bacteria were recovered in greater percentages from the HP feed compared with the BPX.

A Fd × Fm interaction was observed for the sugar-utilizing guild, where the intact BPX showed a larger bacterial population than the defatted BPX (26.3 vs. 7.09%), but populations between the intact and defatted HP, although numerically decreased, were not different (13.6 vs. 6.66%). This was unexpected because the defatting process should remove only the ether extract, leaving all other nutrients in place, relatively increasing their percentages in the feed. However, the lactate-utilizing bacteria trend for form parallels the sugar-utilizing bacteria trend, likely due to cross-feeding (Wolin, 1975). Therefore, it is unclear why defatting the (co)products decreased the sugar utilizing bacterial guilds. However, the population decrease in the sugar-utilizing bacterial guild could account for the slower degradation rates of the defatted (co)products relative to their intact counterparts.

There were no interactions observed for the proteolytic guild. The main effect of feed reiterates the higher protein content of HP compared with BPX by supporting a larger percentage of proteolytic bacteria (35.2 vs. 28.1%; $P < 0.01$). The proteolytic guild also decreased over time in each feedstuff (36.4 vs. 26.9%; $P < 0.01$), and was more prevalent in defatted compared with intact samples (26.1 vs. 37.2%; $P < 0.01$). This again is probably because of diminishing substrate over time and the relative increase of substrate upon fat removal.

The lipolytic guild was not affected by feed type, which was unexpected because of differences in the fat levels of the 2 feeds. Percentages of lipolytic bacteria actually increased over time (1.26 vs. 2.49%; $P < 0.01$) and a greater percentage of lipolytic bacteria was recovered from the defatted compared with the intact feedstuffs (2.60% vs. 1.15%; $P < 0.01$). The interaction of T × Fm showed that the defatted forms at 48 h had greater lipolytic populations ($P = 0.02$) than the defatted forms at 24 h and the intact forms at both time points (3.62 vs. 1.58 and 0.94 vs. 1.36%, respectively). These findings were contrary to what was expected. A possible reason for this increase may be a result of the ability of *Anaerovibrio lipolytica* to degrade aliphatic esters (Henderson, 1971), which may have formed due to lack of fatty acid removal in vitro.

In conclusion, the results of this study indicated that defatting BPX reduced the fermentation rate and lag time before fermentation. We speculated that this result was due to a decrease in sugar-utilizing bacterial populations fermenting the defatted BPX and the lack of lipid inhibition on anaerobic fermentation of fiber. The gas production results were not as explicit for HP. However, defatting both corn (co)products increased fiber-degrading and proteolytic bacterial populations and reduced the pool of available substrate for lactate utilizers. A modified processing method that deoils DDGS may improve feed value and enable greater utilization in dairy cow rations.

CHAPTER IV

AN IN VITRO METHOD FOR DETERMINING SOLUBLE FEED PROTEIN DEGRADATION RATES

INTRODUCTION

Feed protein fractions, designated by their physicochemical properties and degradation rates, help to provide the structure for ration balancing and decision making programs used by the beef and dairy industries (Schwab et al., 2003). However, several researchers have expressed the need to standardize protein determination methods and account for protein fractions often calculated by difference and assigned a fixed assumed degradation rate (Schwab et al., 2003). The in situ method is the most commonly used research method for determining protein degradability in the rumen. However, this method is costly and fails to determine the ruminal degradation rate (**kd**) of the soluble protein fraction, which is known to be variable (120 to 400 %/h) (Sniffen et al., 1992). In vitro methods, however, are a more affordable alternative, less labor intensive and still closely mimic the rumen environment. The major point of concern for rumen fluid in vitro methods is acid accumulation, due to lack of end product removal via passage out of the rumen, but can be overcome by adding adequate buffering salts to the fermentation mixture. Additionally, since no passage occurs, end products are retrievable and thus measureable. Since soluble proteins are rapidly degraded to ammonia by rumen bacteria (Nocek and Russell, 1988), protein degradation rate can be calculated from the rate of ammonia and AA accumulation (Schwab et al., 2003). However, calculation of

protein degradation rates via end products such as ammonia and AA are known to be confounded by microbial uptake for growth (Broderick, 1987). The purpose of the present work was to describe a novel in vitro method, to determine the rate of degradation of the soluble feed protein fraction adjusted for bacterial protein.

MATERIALS AND METHODS

Description of the (co)Products

Four (co)products developed by Poet LLC (Sioux Falls, SD) were utilized to determine the degradation rate of their protein fractions. The first (co)product (Dakota Gold BPX; **BPX-DDGS**) contains added solubles and is the result of a low heat processing and drying method. The low heat method is theorized to lessen the amount of heat-damaged proteins, which are typically found in traditional (co)products. The other (co)products are the result of a novel processing method that physically removes the (Dakota Bran; **BRAN**) and (Dakota Gold Corn Germ Dehydrated; **GERM**) prior to fermentation resulting in a third (co)product (Dakota Gold HP DDG; **HP-DDG**), a high protein product. The solubles from this new processing method are added back to the BRAN and GERM feed products.

Thirty samples (1 kg) of each (co)product (BPX-DDGS, HP-DDG, BRAN and GERM, respectively) were collected and sent to the ruminant nutrition research department at Texas A&M University (College Station, TX). Sub-samples (30 g) were taken and combined to obtain 900g of a composite feed, respectively, for each

Table 4.1: Chemical analysis of 4 corn milling (co)products

Items	Feeds[1]			
	BPX-DDGS	HP-DDG	BRAN	GERM
DM, % as fed	91.5	92.4	90.9	91.4
TDN, % of DM	85.6	79.3	86.5	100.0
CP, % of DM	29.4	43.3	14.6	16.9
Soluble Protein, % of CP	17.2	3.4	34.0	39.7
ADF protein, % of DM	1.4	2.6	1.5	0.9
NDF protein, % of DM	2.8	4.0	1.8	3.2
NFC, % of DM	28.1	24.6	48.8	38.7
Starch, % of DM	6.5	9.7	31.1	25.4
Sugar, % of DM	3.2	1.6	5.3	10.4
Estimated digestibility, % of DM[2]				
RDP (kp = 0.04 h^{-1})	21.2	27.2	12.4	10.7
ADF, % of DM	9.8	11.4	6.8	6.4
NDF, % of DM	29.8	30.0	23.4	24.2
Lignin, % of DM	3.3	3.0	2.0	1.3
Fat, % of DM	10.4	3.9	9.3	17.3
Ash, % of DM	5.1	2.1	5.7	6.1

[1] Feeds analyzed: BPX-DDGS and HP-DDG = corn dried distillers grain (co)products where BPX-DDGS is derived from a low heat processing method and HP-DDG has high protein content, BRAN = corn bran with solubles, and GERM = dehydrated corn germ with solubles. Feeds were composites, homogenized from thirty separately processed batches.

[2] Computed using fractional rate of fermentation of the feed. The estimated fractional rate of passage (kp = 0.04 h^{-1}) is based on typical diets of dry cows, as predicted by the CPM-dairy Model (Boston et al., 2000).

(co)product. Composite feed samples were then sent to Cumberland Valley Analytical Service (Hagerstown, MD) for chemical analysis. Chemical analyses of the corn (co)products are shown in Table 4.1.

Fermentation Method and Sample Collection

There were four treatments, where each (co)product (**Fd**) was combined with a rumen fluid (**Rf**) buffered media (**Md**) mixture (**Fd+Rf+Md**). Three controls were used: C1) (**Fd+Md**), where (co)products were mixed with buffer media, respectively, to account for protein solubility upon saturation of the feed; C2) (**Rf+Md**), where rumen fluid was mixed with media to account for any pre-existing nitrogen and bacterial protein in the inoculate; and C3) (**Md**), where buffering media was incubated to account for any endogenous nitrogen. Each treatment or control was incubated in duplicate (n=20).

Composite feeds were hand ground using mortar and pestle to pass a 2 mm screen, (0.60 g) transferred into 125 mL Wheaton bottles and dampened with 6.0 mL of distilled water (to prevent feed particle scattering). Bottles were flushed with CO_2 to create an in vitro anaerobic atmosphere, and 42 mL of Goering and Van Soest's (1970) buffer media was added. Bottles were closed with butyl rubber stoppers, crimp sealed and placed in a shallow 39 ^{o}C water bath. Rumen fluid from a non-lactating Jersey cow, grazing medium quality grass and receiving a balanced salt and mineral supplement, was collected and filtered through 8 layers of cheesecloth and continuously flushed with

CO_2. Rumen fluid pH was recorded and 12 mL of filtered inoculate was injected via 20G needle and syringe, into appropriate bottles.

Seven time points were used to collect fermentation products (0, 1, 3, 6, 12, 24 and 48 h of fermentation) for analyses. The T0 sample was collected from the bottles, immediately following inoculation. Fermentation product samples were collected by removing 4 mL of fluid from each treatment, with needle and syringe. Collected samples were transferred to micro-tubes and centrifuged at 10,000 × *g* for 5 minutes to remove microbial debris. Cell-free supernatant was then transferred into 4 mL tubes and stored at -20 ^{o}F, to await further analysis. The residual pellets containing the microbial mass were re-suspended in NaCl, to prevent cell shattering, and also stored at -20 ^{o}F.

Ammonia and Bacterial Protein Determination

Ammonia concentration of the supernatant samples were determined by the method of Chaney and Marbach (1962), and assayed in duplicate. The Bradford method (Bradford, 1976) was used, in microtiter plate format, and compared with a BSA standard to determine protein content of the bacterial pellets. Immediately prior to the procedure, bacterial pellets were lysed with 500 µl of 1 M NaOH and centrifuged (10,000 × *g* for 5 minutes), to allow for the solubilization of membrane proteins and reduce protein-to protein variation in color yield (Stoscheck, 1990), and resulting supernatant was assayed.

Enumeration and Statistical Analysis

Bacterial protein for each feed was calculated by difference of the controls from the fermentation mixture, at each time point. The average of the Fd + Md (C1), Rf+Md (C2) and Md (C3) control replicates where used for each feed's calculation. Therefore, the following equation [Eq.1] was used to determine either, 1) Ammonia production from bacteria degrading the (co)product, or 2) Soluble protein consumed by the bacteria degrading the (co)product.

$$\text{Bacterial protein} = Trt_j - (C1_j - C3) - (C2 - C3) - C3 \qquad \text{[Eq. 1]}$$

where, Trt is the buffer media mixed ruminal fluid fermentation of the j^{th} (co)product, C1 is the feed and buffer media mixture of the j^{th} (co)product, C2 is the average of the rumen fluid and buffer media mixture replicates, and C3 is the average of the buffer media replicates.

Actual specific activity of ammonia production (**ASAAP**) was calculated as the change in ammonia production over the change of bacterial protein, between the first and third hours of fermentation ($nmol \cdot mg\ protein^{-1} \cdot min^{-1}$) [Eq. 2].

$$ASAAP_j = [\Delta\ NH_3\ (T3 - T1) / \Delta\ \text{bacterial protein}\ (T3 - T1)] / 120 \qquad \text{[Eq. 2]}$$

where ASAAP is the actual specific activity of the j^{th} feed, $\Delta\ NH_3$ (T3 – T1) is the change in ammonia concentration (nM) between the third and first hour of fermentation, Δ bacterial protein (T3 – T1) is the change in bacterial protein concentration (mg/L)

between the third and first hour of fermentation, all divided by 120 minutes of fermentation.

The fractional rate of ammonia disappearance (kf, 1/h) was obtained for the post-ammonia peak using the PROC NLIN of SAS (SAS Inst. Inc., Cary, NC) as shown in Eq. 3.

$$kf = NH_{3,t=0} \times \exp(-kf \times t) \quad \text{[Eq. 3]}$$

where $NH_{3,t}$ is the ammonia concentration (nM) at time t and kf is the fractional rate of disappearance of ammonia.

The ASAAP, peak ammonia production, and fractional rate of ammonia disappearance was analyzed using PROC MIXED of SAS version 9.2 (SAS Inst. Inc., Cary, NC) using the Least Squares Means for multiple comparisons of the (co)products.

RESULTS AND DISCUSSION

Ammonia Production and Specific Activity

Table 4.2 shows the ammonia activity of the four corn milling (co)products. Although numerical differences were evident, no significant differences existed between feeds for ASAAP (723 ± 5.66 nmol·mg protein^{-1}·min^{-1}), peak ammonia production (1495.3 ± 239.6 nM) or fractional rate of ammonia disappearance (0.12 ± 0.03 nM·min^{-1}) likely due to the large variation between replicates.

Although not significantly different from the other (co)products, the ASAAP was lowest (0 nmol·mg protein^{-1}·min^{-1}) for GERM. This result may be due to the difference

Table 4.2: Ammonia activity of four corn milling (co)products and post analysis of pH measurements

Item[1]		Feeds[2]					
	n	BPX-DDGS	HP-DDG	BRAN	GERM	SEM	*P*-value
ASAAP, nmol/mg protein^{-1}/min^{-1}	8	14.17	4.18	10.57	0.00	5.66	0.40
Peak, nM	8	1344.10	797.99	2097.76	1741.17	239.60	0.07
kf, nM/min^{-1}	8	0.06	0.13	0.13	0.15	0.03	0.30
t, min	8	360.0	60.0	360.0	360.0		
pH [3]	140	6.51^{a}	6.49^{a}	6.27^{b}	6.29^{b}	0.06	<0.01

[1] ASAAP = actual specific activity of ammonia production, Peak = primary ammonia peak, kf = rate of ammonia disappearance, t = time to primary ammonia peak in minutes, pH = pH measurement of (co)product fermentation after 48 h.
[2] Values are least squares means (LSM) and SEM is the average of the standard error of the mean. BPX-DDGS and HP-DDG are corn dried distillers grain where the primary is derived from a low heat processing method and the latter has high protein content. BRAN = corn bran with solubles, GERM = corn dehydrated germ with solubles.
[3]Values are pH measurements taken after 48 h of fermentation from previous research by Tedeschi et al. (2009) evaluating similar (co)products. A post analysis was performed of the pH measurements not reported in that study.

in lipid content of the feeds. Sun et al. (2007) reported that fats have been used to protect amino acids from microbial degradation in the rumen. The chemical analysis of the (co)products revealed that GERM contained the greatest percent fat, followed by BPX-DDGS, BRAN and HP-DDG (17.3 % vs. 10.4, 9.3 and 3.9 %, DM, respectively). However, HP-DDG had the second lowest ASAAP, conflicting with this hypothesis. The ASAAP of the HP-DDG may have been more affected by its initial soluble protein content. Comparatively, HP-DDG had the least amount of soluble protein of the (co)products (1.47 vs. BPX-DDGS = 5.06, BRAN = 4.96, and GERM = 6.73, % of DM). This may also explain why HP-DDG had, numerically, the lowest peak ammonia accumulation (797.99 nM).

Conversely, peak ammonia production was numerically greatest for the BRAN (co)product. Ammonia can accumulate when bacteria ferment amino acids releasing NH_3 and carboxylic acids when energy is limiting, or the rate of protein degradation is greater than the rate of CHO degradation (Nocek and Russell, 1988). Therefore, this result was unexpected considering that the BRAN (co)product contained the greatest percent NFC (48.8%, DM), which is readily fermentable. This outcome can neither be explained by fat content or soluble protein content. Having recognized the NFC content as being nearly 50 %, it was thought, although not measured in this research, that the fermentation may have been effected by a drop in pH. However, Lana et al. (1998) reported that a reduction in ruminal pH would decrease ammonia production. Tedeschi et al. (2009) reported fermentation rates of similar corn milling (co)products, where GERM (0.196 h^{-1}) had the fastest fractional rate of fermentation, followed by BRAN

(0.17 h^{-1}) BPX-DDGS (0.165 h^{-1}) and HP-DDG (0.151 h^{-1}). BRAN was also reported to produce the most total gas of the (co)products and started fermenting sooner than GERM and HP-DDG (Tedeschi et al., 2009). Moreover, BRAN and GERM were concluded to be fed in energy limiting situations, over HP-DDG and BPX-DDGS. Analysis of pH at 48 h was not reported by Tedeschi et al. (2009), but preliminary analysis of their data revealed significant differences between final pH of the feeds, and shown in Table 4.2. The BRAN and GERM had a much lower final pH than the BPX-DDGS and HP-DDG. Still, the preliminary results reported by Tedeschi et al. (2009) of the BRAN fermentation characteristics and the post analysis of pH do not explain why BRAN produced the largest pool of ammonia. Therefore we speculate that this outcome could be a result of a difference in the soluble protein constituents of the (co)products. During the corn milling process, BPX-DDGS and HP-DDG undergo fermentation, and inherit the yeast in the final product. These cells may be over estimating the soluble protein fraction of the resulting (co)products. If this is true, then the soluble protein fraction in BRAN and GERM may have a faster degradation rate than the two fermented (co)products, which may have exceeded the CHO degradation rate, causing greater ammonia production.

There was no difference of the fractional rate of ammonia disappearance, although numerically, the rate for BPX-DDGS was less than half of HP-DDG, BRAN and GERM. This was likely due to the large variation between replicates of BPX-DDGS at 48 h.

Evaluation of the Methodology

The methodology herein was based on the hypothesis that bacterial uptake of protein could be accounted for using different fermentation controls and by measuring bacterial protein. The first control (C1) was to account for protein that is soluble in neutral liquid media. It is important to correct for this as saturation of feed can release soluble protein at varying rates, and solubility is not equal to degradation (NRC, 2001). The second control (C2) was to correct for soluble protein in the rumen inoculate and microbial protein. The third control (C3) was to account for any protein detected in the buffering media from the casein, the nitrogen source in the Goering and Van Soest's (1970) media. By difference, the resulting ammonia production and bacterial protein measurements should be a direct result of the fermentation of the (co)products.

The ASAAP of the (co)product fermentations were compared to the results of other in vitro studies, by Russell et al. (1988),Yang and Russell (1993) and Eschenlauer et al. (2002), to gauge the legitimacy of our results. The previous researchers reported specific activity of ammonia production of mixed ruminal microorganisms varied from 1.8 to 30 nmol·mg protein^{-1}·min^{-1}, depending on the substrate and amount being fermented. Eschenlauer et al. (2002) used several substrates at concentrations between 2 and 20 mg/mL and reported rates of NH_3 production between 1.8 and 19.7 nmol·mg protein^{-1}·min^{-1}. The present study used a substrate (actual (co)products) concentration of 10 mg/mL and resulted in rates between 0 and 14.77 nmol·mg protein^{-1}·min^{-1}. These results suggest justifiable outcomes using the current method.

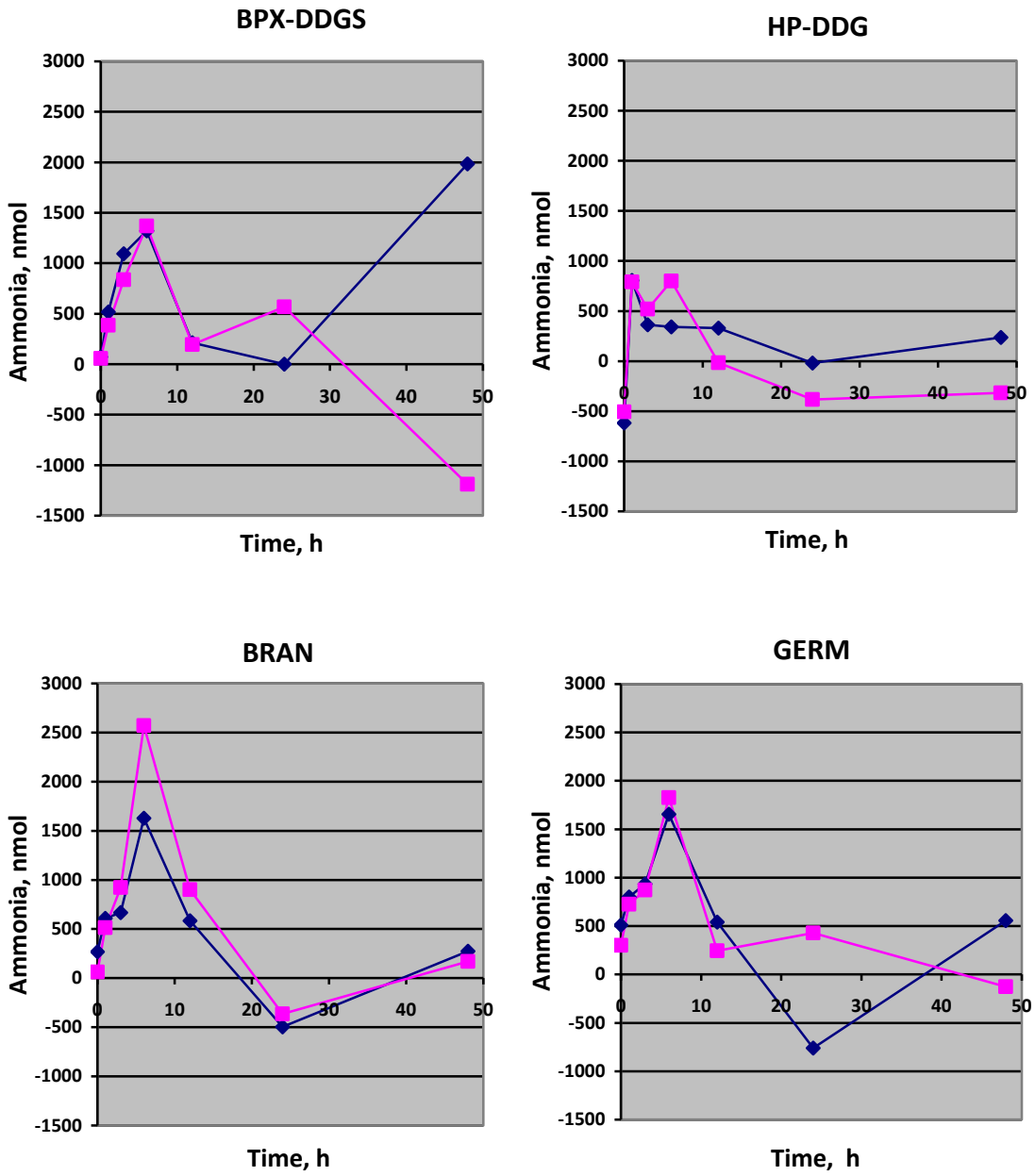

Figure 4.1: Ammonia production of corn milling (co)products fermented in vitro adjusted for bacterial protein. Negative values indicate microbial protein synthesis. BPX-DDGS and HP-DDG are corn dried distillers grain where the primary is derived from a low heat processing method and the latter has high protein content. BRAN = corn bran with solubles, GERM = corn dehydrated germ with solubles. Each point is the mean of two replicate samples within one fermentation. Each feed was fermented in replicate (rep 1,◆; rep 2 ■).

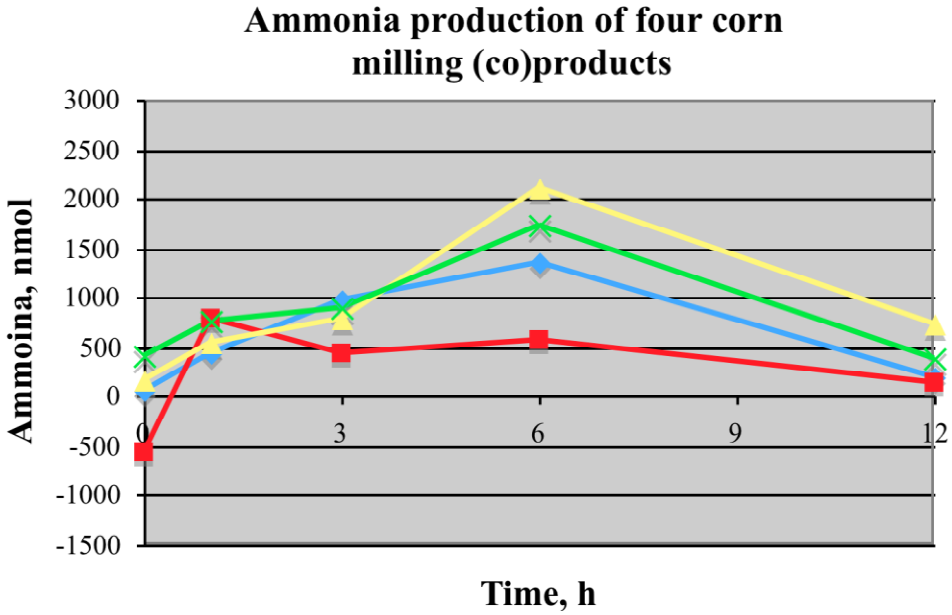

Figure 4.2: Average ammonia production of replicate corn milling (co)products fermented in vitro adjusted for bacterial protein. Negative values indicate microbial protein synthesis. BPX-DDGS and HP-DDG are corn dried distillers grain where the primary is derived from a low heat processing method and the latter has high protein content. BRAN = corn bran with solubles, GERM = corn dehydrated germ with solubles. Each point is the mean of two replicate samples within one fermentation. Each feed was fermented in replicate (BPX-DDGS,◆; HP-DDG ■; BRAN ▲; GERM □).

Variation of replicates. It was evident by the reported standard errors that large variation existed between replicates, even after feed samples had been ground and homogenized. After preliminary analysis, it was clear that more replicates were needed to account for variation between fermentations. Variation between ammonia production profiles of the replicates over time in Figure 4.1.

For BPX-DDGS and GERM, variation was most explicit after Time 12 of fermentation, which was expected due to the nature of the soluble protein fraction. For the purposes of this study incubation times should be limited to less than 12 h. Additionally, fermentation beyond this point would be subject to interference of the degradation of other protein fractions. The replicates for BRAN showed similarly shaped profiles, but reached very different peaks. The most deviated replicate fermentations belonged to HP-DDG, in which a replicate displayed two distinct peaks early in the fermentation. As stated earlier, the first peak in the profile was considered the primary peak, and is used in Table 4.2. However when replicates were averaged, only one peak was visible. The means of the replicates for each (co)products are illustrated in Figure 4.2. When profiles were negative, it was considered that ammonia produced by the controls was greater than the treatments; i.e., protein was not being degraded to ammonia, but was being synthesized for microbial protein.

Sampling. Samples were taken using 20 gauge needles attached to 5 mL syringes. It was noted that small feed particulate clogged needles, which impeded swiftness of sampling time. It is crucial to be efficient with sampling, especially during the first hours of fermentation when time points are close together. Future research may instead use larger gauged needles or have more than one individual be present to assist during sampling.

Performing assays. Depending on the number of time points, and due to the intricacy of the Chaney and Marbach (1962) ammonia assay, it may be required that multiple persons perform this method to accommodate the faster pace. However,

individuals should be limited to only one station throughout the assay to lessen pipetting error between persons. The Bradford (1976) protein assay proved to be quick and effective yielding results in a matter of minutes and required minimal labor.

In summary, the current method, as it stands, may be ground work for an improved method that determines the degradability of the soluble protein fraction of ruminant feeds. Future research and technology may offer valuable improvements to this method which could evolve into a rapid and reliable routine method.

CHAPTER V

LITERATURE SUMMARY

CORN PROCESSING: WET MILLING

Corn milling (co)products of the ethanol industry, for ruminant consumption, are generated from two main processing methods: wet milling and dry grinding. The wet corn milling process is an intricate process yielding a number of co-products for not only animal, but for human consumption as well, such as corn oil and high-fructose corn syrup. Therefore, the United States wet milling industry is restricted to utilizing only high quality corn of #2 or better, (Stock et al., 1999). The most common (co)products of wet milling used in livestock diets include corn gluten feed, corn germ and corn germ meal, corn bran, and a liquid feed, steep liquor. To begin the process, corn grain is steeped in an aqueous solution of sulfur dioxide and lactic acid (produced by microorganisms) at 50 °C for 24 to 48 hours (Jackson and Shandera, Jr., 1995). As the kernels soften the acidity of the steep water begins to loosen the gluten bonds of the corn, releasing starch. This new corn slurry is then ground to detach the germ and sent to germ separators. After the germ is liberated from the slurry, it is further refined for corn oil extraction. After extraction, the remaining oil-free germ fiber product is commonly used as a livestock feed stuff called corn germ, or further concentrated and finely ground to form a corn germ meal. After germ separation, the remaining slurry continues through a series of grinders and screens to remove the kernel fiber (or bran), as gluten and starch flow through to be collected via centrifuge. The bran may be sold as is or mixed with

concentrated steep water and then dried yielding corn gluten feed (DCGF). Additionally, wet corn gluten feed (WCGF) may be produced by mixing bran and fermented corn extractives (steep liquor) (Allen and Grant, 2000). The steep liquor is concentrated steep water, and may be sold separately as a liquid protein source. The separated gluten is concentrated and dried to form the high protein feed corn gluten meal, while the remaining starch component is used to produce ethanol and sweeteners.

CORN PROCESSING: DRY GRINDING

Ethanol may be produced by either wet or dry process, but is primarily a product of dry grinding. Hence, corn milling (co)products from this method have become increasingly available congruently with the demand for ethanol. Three primary feeds produced by the dry grinding industry are distillers' grains (wet or dried), distillers' grains plus solubles (wet or dried), and condensed distillers' solubles. In this process, whole shelled corn is ground by either hammer or roller mills to create a corn meal that is easily saturated during the subsequent cooking process (Rausch and Belyea, 2006). Water and amylase are added to the corn meal and cooked to form liquefied slurry. A yeast culture is then added to ferment the starch into ethanol and CO_2, leaving the proteins, fats, and fiber as intact solids. The ethanol and CO_2 are extorted leaving what is called whole stillage. From there, a centrifuge separates the larger wet grains from the liquid portion, or thin stillage. The wet grain may be sold as wet distillers' grain (**WDG**) or may continue on to a rotary drum dryer resulting in dried distillers' grains (**DDG**). The thin stillage is ushered to an evaporator and then condensed to form a syrup, called

condensed corn distillers solubles (**CCDS**). Solubles may be sold to be used as livestock feed additives or may be mixed back into the wet grains to form wet corn distillers' grain plus solubles (**WDGS**) or dried alongside the wet grains in the rotary drier to yield dried distiller grains plus solubles (**DDGS**).

NOVEL PROCESSING TECHNIQUES

New technologies and processing methods have been utilized to improve the nutritional value of corn milling (co)products and increase ethanol production efficiency. One such process used by Poet LLC (Sioux Falls, SD), modifies the dry grind process by eliminating the cooking step prior to fermentation and implementing raw starch hydrolysis in its place (Wang et al., 2007), resulting in Dakota Gold BPX (**BPX-DDGS**). By eliminating this heating step, the end corn milling (co)product is less likely to have heat damaged proteins, which are known to have lower digestibility in ruminants (Krishnamoorthy et al., 1982). Solubles are added back to this product, making BPX a highly digestible, high energy feed stuff.

Another process of Poet LLC involves a fractionation process prior to fermentation, similar to the beginning stages of wet milling, resulting in three value-added feed stuffs. In this process, the bran (Dakota Bran; **BRAN**) and germ (Dakota Gold Corn Germ Dehydrated; **GERM**) components are removed and the remaining nutrient components go on to be fermented, as in traditional dry grinding, and further processed yielding Dakota Gold HP DDG (**HP-DDG**). The resulting HP is high in protein (42%, DM basis) and NDF (24%, DM), making it a suitable all-in-one energy

and protein supplement for grazing cattle or a more economic corn replacement for total mixed ration (**TMR**) feed strategies. The solubles extracted and concentrated from this process are added back to the BRAN and GERM (co) products.

NUTRIENT COMPOSITION OF CORN (CO)PRODUCTS AND INCLUSION IN DAIRY COW RATIONS

The primary objective of the dairy industry is to provide a cost effective ration to dairy cows that meets energy and protein requirements for lactation and optimizes the genetic potential for milk production, without sacrificing milk quality. Factors that effect ME and MP requirements include BW, parity, phase of lactation, and production level. As milk production level increases, so must the animal's DMI to meet the increasing nutrient demands. Factors of dairy cow diets that influence intake and milk production include; forage-to-concentrate ratio (**F:C**), type and proportion of energy and protein sources, degree of feed processing, and proper mixture of ingredient particle size in TMR. It is recommended that physically effective fiber be included at 22 % of the ration for optimal milk fat synthesis and prevention of rumen epithelial damage (Mertens, 1997). Using this guideline, rations may contain up to 40 % forage, depending on nutrient content. Dairy cow rations have thus turned to nutrient dense feedstuffs to meet energy and protein demands to offset the bulk density of the effective fiber required in the diet.

Ground corn has been the primary source of highly fermentable carbohydrates to meet the energy demands of dairy cattle. However, due to the increase in corn prices as a

response to the demand for clean burning bio-fuels, combined with economic recession, some dairy producers have turned to the more economical and readily available nutrient dense corn ethanol by-products. The replacement value of dried distillers grain, with or without solubles (**DDG(S)**) compared to processed corn lies not only in the ability to provide a cheaper alternative of energy and protein, but also in the long term effects of utilizing corn (co)products in a feeding program. Replacing corn with DDG(S) may also reduce economic losses by lessening the occurrence of sub-acute and acute ruminal acidosis and its detrimental effects on milk production and quality. Replacing corn with DDG(S) dilutes the starch pool in the rumen.

Corn grain is approximately two thirds starch and one third NDF, protein, fat, and ash. In each processing method, the corn starch is removed at some point, increasing the concentration of the remaining nutrients three fold. According to the NRC (2001), corn grain typically has low CP and RUP content of 9.4 % and 3.6 % DM, respectively, while DDG(S) have CP and RUP of 29.7 % and 8.2 % DM. The NRC (2001) provides a feed library listing the chemical composition for many corn milling co-products, and is updated approximately every ten years. Although the feed library provides a valuable source of information, the data given is based on approximations of the chemical analysis of samples and cannot account for the entire population of all feed. This can be the cause for concern for buyers as it is known that processing methods differ from plant to plant (Spiehs et al., 2002) as well as within a single ethanol plant (Belyea et al., 2004). In addition, the values listed in typical feed libraries does not represent a real feed (Tedeschi et al., 2002; 2005). Therefore, even if the feed library values are used, the

values may be greater or less for any given batch of (co)product. However, with more research gearing to expand the feed library, this range of variation will decrease over time, due not only to increased nutrient analysis data available, but also improved and more consistent processing technology.

Aside from nutrient variability across products, higher fat, phosphorus and sulfur content may limit the use of DDG(S) in a TMR. Table 5.1 compares the nutrient content of corn to DDG(S). The phosphorus content of DDG(S) can be three times greater and can require added supplements to a TMR, such as limestone, to balance proper Ca:P ratio. Although feeding higher levels of these minerals should not impact the health or milk production of ruminants, it may be concerning from an environmental stand point. Land application of manure with greater mineral concentrations may contaminate surface and ground water, if not managed properly. The amount of solubles added back to DDG(S) may also be a limiting factor due to the detrimental effects of fat on fiber digestion (Van Soest, 1994) and milk quality (Pentoja et al., 1994). Research has reported unfavorable milk characteristics as a result of feeding unsaturated fat (soybean oil) in dairy cow rations (Macleod and Wood, 1972). Combined with variation in fat content (10-12%) balancing rations that include large percentages of DDG(S) may be subject to the negative effects of plant oils, such as decreased milk fat and protein. For these reasons, the inclusion of DDG(S) in dairy cow diets is sometimes limited.

However, research has been conducted to determine optimal inclusion rates for dairy cow rations. Some studies have reported favorable findings when including DDG(S) in rations as a protein source instead of soybean meal in which milk production was either

unaffected (Owen and Larson, 1991) or increased (Powers et al., 1995; Nichols et al., 1998). These studies suggested an optimal inclusion of 15- 20% of the ration on a DM basis. However, more recent studies have indicated levels of up to 30% of dietary DM can be fed to lactating dairy cows, resulting in greater DMI, milk yield, milk protein and fat yields (Janicek et al., 2008). Therefore, it is important to realize that optimal inclusion rates of DDG(S) are ultimately dependent on various dietary and economic considerations.

Table 5.1: Chemical analysis of corn and dried distillers grain with or without solubles

	Feeds[1]				
Item	Corn, dry, ground	DDGS 1	DDGS 2	BPX-DDGS	HP-DDG
DM, %AF	88.1	92.0	90.2	91.1	92.1
NE_L, Mcal/kg	2.01	2.04	1.97	2.27	2.27
CP, %DM	9.4	25.0	29.7	28.3	42.0
NDF, %DM	9.5	44.0	38.8	26.1	24.5
ADF, %DM	3.4	18.0	19.7	9.9	12.7
Ether extract, %DM	4.2	10.3	10.0	11.4	4.1
Ca, %DM	0.04	0.15	0.22	0.05	0.02
P, %DM	0.30	0.71	0.83	1.04	0.46

[1]Corn,dry, ground and DDGS 2 feed values obtained from NRC (2001), DDGS1 feed values obtained from NRC (1989), BPX-DDGS and HP-DDG are corn dried distillers grain (co)products in which BPX-DDGS undergoes a low heat process and HP-DDG has high protein content (Poet Nutrition LLC (Sioux Falls, SD).

NUTRITION MODELS AND THE CORNELL NET CARBOHYDRATE AND PROTEIN SYSTEM

Nutrition models are an invaluable aid to help producers maximize business potential. Computerized support systems help make decisions based on model simulations. Although model output is not always accurate or precise, their strength lies in understanding their weaknesses. Testing model output with observed animal performance provides vital knowledge for revisions, enabling models to become more powerful (Tedeschi, 2006). Numerous systems have been utilized in the last century in attempt to predict energy and protein availability. Initially, the Weende and total digestible nutrients (**TDN**) systems, based on proximate analysis, were used to assess feed value (Van Soest, 1967). From these models, subsequent net energy (**NE**) systems were developed to adjust for methane, urinary and heat increment losses (Sniffen et al., 1992; NRC, 1978). Under standard feeding conditions, the NE system can accurately predict net energy of individual feeds but overlooks animal variation and divergent feeding conditions (Sniffen et al., 1992). Hence, more intricate systems have been developed to more precisely predict NE and protein availability and also account for fermentation and passage of individual feed fractions. Currently, the model most widely used in the dairy industry is the Cornell Net Carbohydrate and Protein System (**CNCPS**).

The CNCPS divides plant carbohydrates and proteins into sub-categories based on physicochemical characteristics, ruminal degradation, and post-ruminal digestion characteristics (Sniffen et al., 1992). The CNCPS uses feed carbohydrate and protein

degradation and passage rates to predict extent of ruminal fermentation, microbial protein production, post-ruminal absorption and total supply of metabolizable energy (**ME**) and protein to the animal (Fox et al., 2004). Acknowledging differences in nutrient degradation and utilization promotes the accuracy of the CNCPS, whereas previous models had not established such variables.

Additionally, the CNCPS allows inputs for specific farm management practices, environmental conditions, animal variables, feed composition characteristics, and management techniques when formulating rations to more accurately predict growth and milk production while minimizing nutrient excretion to the environment (Fox et al., 2004). Furthermore, the CNCPS includes several sub-models which adjust for the physiological functions of maintenance, growth, pregnancy, lactation, reserves, feed intake and composition, rumen fermentation, intestinal digestion, metabolism, and nutrient excretion (Fox et al., 2004). The NRC (2001) recognizes the proficiency of the CNCPS and has adopted several CNCPS sub-models. The CNCPS has also given way to a commercially available program developed by researchers at Cornell University, University of Pennsylvania, and the Miner Institute, known as the Cornell-Penn-Miner Institute (**CPM**) Dairy Model (http://www.cpmdairy.net/Index.php). Its versatility and extensive feed library has made it invaluable to dairy nutrition consultants and feed companies, stimulating the industry's interest in more research in model efficiency.

CARBOHYDRATE FRACTIONATION OF THE CNCPS-BASED NUTRITION MODELS

Feed carbohydrates can be divided into two categories; fiber (**FC**) and non-fiber carbohydrates (**NFC**) (Fox et al., 2004). Total carbohydrate concentration of a feedstuff can be calculated by difference if the total crude protein, fat, and ash contents are known using the equation, 100 – CP – Fat – Ash (Sniffen et al., 1992). Total FC is equivalent to the cellulose and hemicellulose found in the neutral detergent fiber (**NDF**), and NFC is the difference of total DM and NDF of the feedstuff (Fox et al., 2004). The NFC constituents (starch, pectin, and sugar) are soluble in neutral detergents (Sniffen et al., 1992). Feedstuffs have different rates of degradation in the rumen depending on the type and proportion of carbohydrate they contain. Moreover, responses to either type of carbohydrate being fermented can significantly alter fermentation end product production and subsequent animal performance (Russell et al., 1992).

The CNCPS separates total carbohydrates into three different fractions, A, B, and C, based on degradation rates in the rumen. The fractions can be calculated if the chemical entities of nonstructural carbohydrates **(NSC)**, structural carbohydrates **(SC)**, and indigestible fiber are known (Sniffen et al., 1992). Fraction A is water soluble (Fox et al., 2004) and fermented very rapidly in the rumen at about 300 %/h and includes silage acids, sugars, other organic acids, and short oligosaccharides (Sniffen et al., 1992). Fraction B is sub-divided into B1 and B2 with intermediate availability in the rumen and is comprised of starch and pectin fermenting anywhere from 2 to 50 %/h (Sniffen et al., 1992).

Corn milling (co)products vary in starch content depending on the milling process from which they are produced and may contain zero to 5% starch for distillers grains (Murthy et al., 2009), 11.2% starch for high protein distillers grains, and 23.6% starch for corn germ meal (Widmer et al., 2007). The fermentation rate of starch is highly dependent on how feedstuffs are used, processed, and stored (Russell et al., 1992). Cereal grains that have been ground to decrease particle size and ensiled forages will have more rapid starch and pectin fermentation by rumen microbes (Sniffen et al., 1992.). Carbohydrate fraction B1 is fermented in the rumen by rapidly growing microbial populations that utilize ammonia or peptides as a nitrogen source (Van Soest, 1982; Russell et al., 1992). Fraction B2 is slowly degraded in the rumen by bacteria that only utilize ammonia to obtain their necessary nitrogen requirement and includes the available cell wall components of soluble fiber and plant acids (Russell et al., 1992; Sniffen et al., 1992). Mean digestion rate of the B2 fraction is digested at 5.1 %/h for mature grains (Smith et al., 1972), 4.8 to 5.4 %/h for feedstuffs that contain 50-60% CP (corn gluten meal, soybean meal, and peanut meal; Varga and Hoover, 1983), and 6.5 to 7.2 %/h for protein sources that contain 25-30% CP (distillers grains, corn gluten feed, and brewers grains; Varga and Hoover, 1983).

Fraction C is unavailable to rumen fermentation and includes lignin bound fiber. Although lignin is not a carbohydrate, the C fraction is equivalent to lignin content as a percent DM multiplied by 2.4 of the leftover material following a 72 hour *in vitro* incubation (Smith et al., 1972; Mertens, 1973). Lignin concentration can vary widely depending on type and maturity of the feedstuff. According to the NRC (2001), the

lignin concentration for dried distillers' grain is 4.3% of DM. Sniffen et al. (1992) provided the following equations to calculate the carbohydrate fractions:

CHO_j (% DM) = 100 – CP_j (% DM) – Fat_j (% DM) – Ash_j (% DM);

CC_j (% CHO) = 100 × [NDF_j (% DM) × 0.01 × $Lignin_j$ (% NDF) × 2.4] / CHO_j (% DM);

$CB2_j$ (% CHO) = 100 × [NDF_j (% DM) – $NDIP_j$ (% CP) × 0.01 × CP_j (% DM) – NDF_j (% DM) × 0.01 × $Lignin_j$ (% NDF) × 2.4] / CHO_j (% DM);

$CNFC_j$ (% CHO) = 100 – $B2_j$ (% CHO) – C_j (% CHO);

$CB1_j$ (% CHO) = $Starch_j$ (% NFC) × [100 – $B2_j$ (% CHO) – C_j (% CHO)] /100;

CA_j (% CHO) = [[100 – $Starch_j$ (% NFC)] × [100 – $B2_j$ (% CHO) – C_j (% CHO)]]/ 100;

Where, CP_j (% DM) = percentage of crude protein of the j^{th} feedstuff; CHO_j (% DM) = percentage of carbohydrate of the j^{th} feedstuff; Fat_j (% DM) = percentage of fat of the j^{th} feedstuff; Ash_j (% DM) = percentage of ash of the j^{th} feedstuff; NDF_j (% DM) = percentage of j^{th} feedstuff that is neutral detergent fiber; $NDIP_j$ (% DM) = percentage of neutral detergent insoluble protein of the j^{th} feedstuff; $Lignin_j$ (% NDF) = percentage of lignin of the neutral detergent fiber of the j^{th} feedstuff; $Starch_j$ (% NFC) = percentage of starch in the nonfiber carbohydrate of the j^{th} feedstuff; $Sugar_j$ (% NFC) = percentage of sugar in the nonfiber carbohydrate of the j^{h} feedstuff; CA_j (% CHO) = percentage of carbohydrate of the j^{th} feedstuff that is sugar; $CB1_j$ (% CHO) = percentage of carbohydrate of the j^{th} feedstuff that is starch and nonstructural protein; $CB2_j$ (% CHO) = percentage of carbohydrate of the j^{th} feedstuff that is available fiber; CC_j (% CHO) = percentage of carbohydrate in the j^{th} feedstuff that is unavailable fiber and $CNFC_j$ (%

CHO) = percentage of carbohydrate of the j^{th} feedstuff that is soluble in neutral detergent.

Recently, due to inadequacies in the existing model, Lanzas et al. (2007) proposed some modifications to better fit the degradation characteristics of certain nutrient constituents. Most concerning were the CA and CB1 fractions which are known to have variable degradation rates, especially when processing treatments differ. Additionally, since the CA and CB1 fractions are not routinely analyzed, but are calculated by difference, they inherit errors from the other assays. The expanded model fractionation proposed by Lanzas et al. (2007) further segregated the CA and CB fractions to reflect their digestibility attributes, and were renamed as follows:

CA1 (Volatile fatty acids) = $\text{Acetate}_j + \text{Propionate}_j + \text{Butyrate}_j + \text{Isobutyrate}_j$

CA2 (Lactic acid) = Lactate_j

CA3 (Organic acids) = Organic acids_j

CA4 (Sugars) = Sugars_j

CB1 (Starch) = Starch_j

CB2 (Soluble fiber) = $\text{NFC}_j - \text{CA1}_j - \text{CA2}_j - \text{CA3}_j - \text{CA4}_j - \text{CB1}_j$

CB3 (Digestible fiber) = $\text{NDF}_j - (\text{NDICP}_j \times \text{CP}_j)/1000 - \text{CC}_j$

Although the new fractionation more accurately described the nutrient constituents and their fermentability, the full characterization of feed carbohydrates is limited due to variability in degradation rates of sugar. However, this model may provide an outline for any future methodologies that may better describe the nature and degradation of feed elements that are not currently routinely measured.

PROTEIN FRACTIONATION OF THE CNCPS-BASED NUTRITION MODELS

Feedstuffs contain various proteins and NPN compounds that contribute to the overall CP content. Previously, the nutritive value of CP in a feedstuff was best described by its degradation rate and retention time in the rumen, resulting in two fractions; RDP and RUP (Schwab et al., 2003). However, dividing feed nitrogen into these two categories is inadequate because it does not distinguish NPN from true protein, and does not account for unavailable (bound) nitrogen that is insoluble in acid (Van Soest, 1994). The NRC (2001) recognizes these limitations and instead promotes an in situ technique to declare three different protein fractions. The A fraction includes NPN, soluble protein and protein particulate small enough to pass through the pores of a nylon bag. The B fraction is metabolizable protein dependent of the balance between rate of digestion (**kd**) and rate of passage (**kp**). The C fraction is the unavailable or bound protein. Yet again, NPN and true protein are not distinguished.

The CNCPS model is based on the protein fractionation scheme first described by Van Soest et al. (1981), which categorizes protein fractions according to their solubility in three buffers and their reaction to a protein- precipitating agent resulting in five fractions (Lanzas et al., 2008). In this model, feed protein is divided into NPN, true protein, and unavailable, or bound, protein components which correspond to fractions A, B, and C, respectively (Sniffen et al., 1992). The CNCPS assigns intestinal digestibility coefficients to all fractions and sub-divides the true protein fraction into three categories based on ruminal availability (Schwab et al., 2003).

Fraction A is composed of the NPN compounds, including amines, amides, free amino acids, nucleic acids, nitrate, ammonia, and peptides. These compounds are assumed to be completely converted to ammonia in the rumen (all RDP) and are therefore, assigned an intestinal digestibility coefficient of 100% (Schwab et al., 2003; Sniffen et al., 1992). The A fraction is soluble in borate-phosphate buffer but not precipitated with tungstic acid (Sniffen et al., 1992) and is therefore associated with the liquid portion of ruminal digesta which passes faster than the insoluble, potentially digestible fraction (Nocek, 1988). Estimates of fraction A range from 5.21 to 9.84% CP for distillers grains plus solubles (Kleinschmit et al., 2007).

Fraction B, true protein, is further divided into fractions B1, B2, and B3 based on rates of digestion in the rumen (Lanzas et al., 2008). Fraction B1 contains true proteins, globulins, and albumins that are rapidly degraded in the rumen at 120 to 400 %/h and has an intestinal digestibility coefficient of 100% (Schwab et al., 2003). This protein fraction is soluble in borate-phosphate buffer and is precipitated with tungstic acid (Schwab et al., 2003). Small amounts of both protein and peptides may escape the rumen due to this rapid degradation of protein when peptide and ammonia availability exceed microbial utilization (Sniffen et al., 1992). Estimates of fraction B1 may range from 0.07 to 1.78% CP for distillers grains plus solubles (Kleinschmit et al., 2007).

Fraction B2 consists of true proteins, albumins, and glutelins, and is typically the largest protein fraction in small grains (Van Soest, 1981). The B2 fraction is moderately degraded in the rumen at 3 to 16 %/h and also has an intestinal digestibility coefficient of 100% (Schwab et al., 2003). Corn protein is of poor nutritional quality due to its

deficiency in Lysine and Tryptophan and slower rates of degradation in the rumen (Shukla and Cheryan, 2001). Therefore, the fate of the B2 fraction largely depends on kd and kp in the rumen. This fraction is calculated by difference of total CP and the sum of the other four CP fractions (Schwab et al., 2003). Estimates for protein fraction B2 may range from 40.9 to 51.1% CP for dried distillers grains plus solubles (Kleinschmit et al., 2007).

Fraction B3 contains true proteins, prolamins, such as zein protein in corn, and extensins which are very slowly degraded in the rumen at 0.06 to 0.55 %/h and has an intestinal digestibility coefficient of 80% (Lanzas et al., 2008; Schwab et al., 2003). This fraction is part of the cell wall and is not soluble in neutral detergent but is soluble in acid detergent (Sniffen et al., 1992). The B3 fraction is therefore calculated as the difference between neutral detergent insoluble crude protein **(NDICP)** and acid detergent insoluble crude protein **(ADICP)**. The B3 protein fraction is low in feedstuffs used as protein supplements; however, forages, fermented grains, and corn (co)product feeds are high in B3 (Sniffen et al., 1992). Estimates for protein fraction B3 may range from 22.7 to 41.4% CP for dried distillers grains plus solubles (Kleinschmit et al., 2007).

The protein fraction C represents nitrogen that is highly resistant to microbial and enzymatic degradation and includes lignin-bound nitrogen or protein, tannin-protein complexes, and Maillard products (Sniffen et al., 1992). Fraction C is insoluble in acid detergent and is often referred to as acid detergent insoluble nitrogen (**ADIN**), (ADICP, %DM = ADIN, %DM ×6.25), (Van Soest, 1994). The ADIN content DDG(S) may indicate degree of heat damage to proteins that occurs during the cooking process prior

to fermentation (Firkins et al., 1984). Proteins are denatured by high temperatures; therefore, heat-damaged proteins may have lower solubility and rates of degradation in the rumen and are generally unavailable to digestion (Russell et al., 1992). For most feedstuffs fraction C is assumed to have no intestinal digestibility. However several studies have shown that variable amounts of ADICP can provide amino acids post-ruminally (Schwab et al., 2003; Klopfenstein, 1996; Nakamura et al., 1994). Van Soest (1994) proposed 60% of ADICP in distillers grains is digestible when fed to ruminants. The ADICP may be truly digested, metabolic N in the feces may be reduced, a portion of the ADICP could be absorbed from the small intestine and excreted in the urine, or a combination of these postulations may explain why ADICP does not reflect protein digestibility in distillers' grains (Van Soest, 1994; Kelzer et al., 2010). Estimates for protein fraction C may range from 7.5 to 23.1% CP for dried distillers grains plus solubles (Kleinschmit et al., 2007). Sniffen et al., (1992) reported the following equations to determine feed protein fractions:

PA_j (%CP) = NPN_j (% SOLP) * 0.01 * $SOLP_j$ (% CP);

$PB1_j$ (% CP) = $SOLP_j$ (% CP) – A_j (% CP);

PC_j (% CP) = $ADIP_j$ (% CP);

$PB3_j$ (% CP) = $NDIP_j$ (% CP) – $ADIP_j$ (% CP);

$PB2_j$ (% CP) = 100 – A_j (% CP) – $B1_j$ (% CP) – $B3_j$ (% CP) – C_j (% CP);

Where CP_j (% DM) = percentage of crude protein of the j^{th} feedstuff; NPN_j (% CP) = percentage of crude protein of the j^{th} feedstuff that is non-protein nitrogen × 6.25; $SOLP_j$ (% CP) = percentage of crude protein of the j^{th} feedstuff that is soluble protein; $NDIP_j$ (%

CP) = percentage of crude protein of the j^{th} feedstuff that is neutral detergent insoluble protein; $ADIP_j$ (% CP) = percentage of crude protein of the j^{th} feedstuff that is acid detergent insoluble protein; PA_j (% CP) = percentage of the crude protein of the j^{th} feedstuff that is non-protein nitrogen; $PB1_j$ (% CP) = percentage of crude protein of the j^{th} feedstuff that is rapidly degraded protein; $PB2_j$ (% CP) = percentage of crude protein of the j^{th} feedstuff that is intermediately degraded protein; $PB3_j$ (% CP) = percentage of crude protein of the j^{th} feedstuff that is slowly degraded protein; and PC_j (% CP) = percentage of crude protein of the j^{th} feedstuff that is bound protein.

Although the CNCPS-based models have proved a highly valuable asset to industry users, recent investigators have acknowledged limitations to its scheme. Lanzas et al., (2008) reported these limitations and proposed modifications to the original CNCPS model reported by Sniffen et al. (1992), in effort to increase the power of the protein fractionation model. Briefly, these inadequacies include: 1) assuming insoluble N in neutral detergent and in acid detergent represents slowly degradable (B3) and unavailable protein (C) fractions, respectively, may not be valid for all feeds (Waters et al., 1992; Nakamura et al., 1994; Coblentz et al., 1999); 2) assuming all of the NPN fraction enters the ammonia pool completely and does not provide amino N that can stimulate microbial growth has caused under prediction of microbial protein production (Aquino et al., 2003); 3) assuming fraction A is completely degraded does not account for the contributions of free amino acids and peptides to the RUP flows (Choi et al., 2002; Volden et al., 2002; Reynal et al., 2007); and 4) despite the RUP flow's sensitivity to degradation rates for the B2 fraction, there is no recommended method for

determining B2 rates (Lanzas et al., 2008). The first modification redefined the buffer soluble fractions A and B1 as non-amino acid nitrogen (NAAN) and amino acid nitrogen (AAN), respectively:

$PA'_j = (1{,}000 - AAN_j) \times (SolCP_j/1{,}000) \times (CP_j/1{,}000)$ (g/kg of DM), and

$PB1'_j = (SolCP_j/1{,}000) \times (CP_j/1{,}000) - PA'_j$(g/kg of DM)

where CP_j is the CP content of the j^{th} feed, g/kg DM; AAN_j is the AA N content of the j^{th} feed, g/kg of SolCP; PA'_j is the protein A fraction content of the j^{th} feed, g/kg of DM; $PB1'_j$ is the protein B1 fraction content of the j^{th} feed, g/kg of DM; and $SolCP_j$ is the buffer-soluble CP content, g/kg of CP. The B1′ fraction kp was proposed to pass at the same rate as liquids leaving the rumen.

The second modification involved adjusting the degradation rates of the B1 fraction, as the current CNCPS feed library values exceeded most of the reported values for in vitro soluble proteins (Lanzas et al., 2008). The third modification involved increasing the kd of the B3 fraction which was suggested due to many reports of much greater digestion rates than what was standard in the CNCPS feed library (Lanzas et al., 2008). The final modification was to combine the original B2 and B3 fractions into a solidary fraction, as previous sensitivity analyses indicated negligible differences in degradation rates preventing model predictions from detecting the factions independently (Lanzas et al., 2008). The new fraction was renamed PB2′: $PB2_j' = CP_j - PA_j - PB1_j - PC_j$ (g/kg of DM).

Several schemes were evaluated including assorted combinations of the fore mentioned modifications and compared to the original CNCPS protein fractionation

design. The results indicated that model prediction accuracy of RDP and RUP could be improved by pooling the B2 and B3 fractions of the original CNCPS protein fractionation scheme and assigning a single degradation rate. Lanzas et al., (2008) theorized that describing NDICP (B3) as the slowly degraded protein fraction may be inaccurate. Model prediction accuracy was also improved when the AAN was accounted for in the soluble protein fraction (Lanzas et al., 2008).

Nutrition model predictions will continue to change as researchers persistently challenge the old dogma of the original carbohydrate and protein fractionation schemes. It will be imperative and interesting to assess the accuracy of amended models and their predictions, especially for grain by-products as they become increasingly available.

IN VITRO GAS PRODUCTION TECHNIQUE

The nutritive value of a ruminant feed is determined by the rate and extent of digestion of its chemical components. Determining feed digestibility through in vivo methods is laborious, expensive, requires large quantities of feed, and is impractical for single feedstuffs. In contrast, in vitro methods have been a more convenient and less expensive alternative for feed value determination (Getachew et al., 2004).

Several in vitro procedures have been used to evaluate ruminant feedstuffs including gas-measuring methods. In vitro gas production (**IVGP**) techniques were developed to characterize the fermentation kinetics of ruminant feeds (López et al., 2007). Although gas-measuring methods may vary, all techniques are based on the assumption that the amount of gas produced is directly related to the amount of substrate

being fermented (López et al., 2007; France et al., 2000). Gas production techniques were considered to be a routine feed evaluation method after Menke et al., (1979) reported a high correlation between gas production in vitro and apparent digestibility in vivo. The technique used by Menke et al., (1979) was developed to determine total gas production at 24 h of fermentation using syringes, through the measurement of plunger displacement. However, the current, more widely used technique was adapted from the approach described by Wilkins (1974), in which pressure transducers measure the gas produced from sealed vessel fermentations. Pell and Schofield (1993), Cone et al. (1996), Mauricio et al. (1999), and Davies et al. (2000) further developed this technique to involve automated pressure recording.

In context with the current research, the IVGP technique used was similar to that described by Pell and Schofield (1993) and Schofield and Pell (1995a; 1995b). Briefly, their IVGP technique involves an incubation chamber with a multi-plate stirrer, gas pressure sensors which attach to fermentation bottles, and a software program to record total gas production at regular intervals for a predetermined length of time, typically up to 48 h. The fermentations consist of the feed sample in question, a buffer media and rumen fluid inoculate which are flushed into an anaerobic state and closed with a gas-tight seal. The gas pressure inside each fermentation bottle is measured, recorded and plotted over time by specialized computer software, creating a fermentation profile. The gas production data can then be analyzed for a) total gas production, b) fractional rate of degradation, and c) lag time prior to fermentation (Tedeschi et al., 2009). Several non-linear functions have been developed to conduct kinetic analysis (Schofield et al., 1994).

The function with the smallest sum of squares of error is chosen to represent the fermentation profile. In the case that more than one function fits the data; both model parameters are further compared to distinguish any statistical differences (Williams et al., 2010). Recent research has shown that the most common function used to describe corn (co)products is the discrete exponential equation with lag time (Williams et al., 2010; Kelzer et al., 2010; Tedeschi et al., 2009) and is described by Schofield et al., (1994) and shown in Eq.(1):

$$Y = \begin{cases} a \times (1 - \exp(-b \times (t - c))); \forall t \geq c \\ 0; \forall t < c \end{cases} \quad (1)$$

where a represents the asymptote, mL; b represents the fractional degradation rate, h^{-1}; c represents lag time, h.

This IVGP technique has typically been utilized to describe the fermentation dynamics of whole feedstuffs but has also been useful in evaluating the gas kinetics of feed residues (Williams et al., 2010; Tedeschi et al., 2009) and comparing the gas production dynamics of similar feed types from different processing methods (Williams et al., 2010; Kelzer et al., 2010; Tedeschi et al., 2009).

Several IVGP techniques have been reviewed by Rymer et al. (2005), who indicated that many factors could influence the gas production of a feedstuff. This observation is the result of methodologies evolving from different laboratories. Factors reported to have significant effect on gas production included ratios of buffer to substrate being fermented, weighing error, fermentations lasting longer than 48 h, and most importantly, variation of inoculum source. Other less influential factors included

substrate particle size, agitation during the fermentation process. Although a standardized IVGP technique is not available, the current techniques still remain useful tools to characterize the fermentation dynamics of common ruminant feedstuffs.

MOLECULAR IDENTIFICATION OF RUMEN BACTERIA

It is accepted that the primary role of the rumen is to digest roughages for energy, which are largely unusable by monogastrics (Van Soest, 1994). The digestion of cellulosic matter can be attributed to the anaerobic bacteria that inhabit the rumen. Nutrients ingested by the animal are digested by microbes resulting in fermentation gases, which are expelled via eructation, and volatile fatty acids (**VFA**) which are absorbed across the rumen wall and are the primary energy source for ruminants. Additionally, enzymes secreted by the microorganisms break down proteins, and hydrolyze exogenous fats which may later be absorbed by the small intestine. However, the bacterial habitat of the rumen is very diverse and bacterial populations may shift in response to changes in animal diet, or as a result of acidic fermentation end-product concentrations in their environment. Hungate et al., (1964) initially proposed that bacterial population diversity depends on available substrate in the rumen and that the bacterial habitat would select for species that would generate the most 'biochemical work' via cross-feeding of intermediate end-products. Therefore it is of great interest to research the nutrient requirements of rumen microbes to best benefit the host.

The study of anaerobic bacteria was first made possible through the isolation of a few bacterial species using Hungate's (1950) 'role tube technique'. Until then, strictly

anaerobic bacteria had not been studied due to a lack of understanding of oxygen toxicity and their sensitive requirements for survival (Van Soest, 1994). Cultivation of anaerobic bacteria unlocked the door to understanding the fermentation process of the cellulosic diets of ruminants. At the time, bacteria were categorized by their morphological characteristics, substrate affinities, and end-products, into taxonomic groups (Russell, 2002). However, as more bacteria were isolated, several researchers noted that although these characteristics were useful for identification, it was not a solid basis for the classification of bacteria. In general, morphology may vary considerably within bacterial species as well as between different cultures of the same strain, and don't necessarily reflect phylogenetic relationships (Dworkin et al., 2006, Mannarelli et al., 1991). Church (1988) acknowledged considerable overlapping of bacteria when assigning bacteria to substrate niches because many of the predominant bacteria are capable of utilizing more than a few different substrates for energy.

Although bacterial diversity in the rumen has been well documented by Hungate (1966), Church (1988), Russell (2002), and Dehority (2003), as well as many others, microbiologists speculate that only about 10% of rumen bacteria have actually been cultivated. Around the same time Hungate (1950) was introducing the roll tube technique research discovered that DNA was the molecule responsible for genetic variation (Russell, 2002). However the application of this knowledge would not be routinely used for several decades. First, bacterial relationships were determined by DNA hybridization, where a single strand of known bacterial DNA binds to a single strand of unknown bacterial DNA: the greater the complementary binding of the strands,

the closer the genetic relationship. Mannarelli et al. (1991) went on to reexamine the taxonomic relationships of some rumen bacteria, which were first classified in the 1950's by Bryant et al. (1958) based on nutritional requirements. With new techniques available Mannarelli et al. (1991) reported a low DNA homology between 14 strains of *Bacteroides ruminicola* suggesting a reclassification of at least 6 of the strains.

Currently, most of what is known about bacterial phylogeny is based on 16S rRNA and corresponding rDNA gene sequences (Russell, 2002). One current method used to identify bacterial DNA is the 16s rDNA bacterial tag encoded FLX amplicon pyrosequencing (**bTEFAP**) technique. This bTEFAP technique has been utilized to identify malignant bacteria of infected wounds and surgical sites (Wolcott et al., 2009a; 2009b; Dowd et al., 2008b), and those in animal feces that pose potential risk to human health (Dowd et al., 2008a; 2008c). However, Williams et al. (2010) recently utilized the bTEFAP technique to observe population shifts of bacteria, categorized by substrate niche, fermenting intact or modified ruminant feedstuffs. The nutrition community may benefit from the bTEFAP technique when examining effects of feedstuff processing, or testing genetically altered feedstuffs and their effects on rumen bacteria. Additionally, similar techniques may also help to identify and classify unknown, or previously uncultured rumen bacteria that may share genetic similarities with known rumen bacteria species. In time, with such tools available, it may be possible to discover and classify a large portion of the existing rumen microbes.

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