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W. L. Williams
Texas A&M University

L. O. Tedeschi
Texas A&M University, luis.tedeschi@tamu.edu

Paul J. Kononoff
University of Nebraska-Lincoln, pkononoff2@unl.edu

T. R. Callaway
USDA-ARS, todd.callaway@ars.usda.gov

S. E. Dowd
Medical Biofilm Research Institute and Research and Testing Laboratory

See next page for additional authors

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Evaluation of in vitro gas production and rumen bacterial populations fermenting corn milling (co)products

W. L. Williams,* L. O. Tedeschi,*[1] P. J. Kononoff,† T. R. Callaway,‡ S. E. Dowd,§ K. Karges,# and M. L. Gibson#[2]
*Department of Animal Science, Texas A&M University, College Station 77843-2471
†Department of Animal Science, University of Nebraska-Lincoln, Lincoln 68583-0908
‡Food and Feed Safety Research Unit, USDA-ARS, College Station, TX 77845
§Medical Biofilm Research Institute and Research and Testing Laboratory, Lubbock, TX 79410
#Dakota Gold Research Association, Sioux Falls, SD 57104-4506

ABSTRACT

The objective of this study was to evaluate the fermentation dynamics of 2 commonly fed corn (co)products in their intact and defatted forms, using the in vitro gas production (IVGP) technique, and to investigate the shifts of the predominant rumen bacterial populations using the 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) technique. The bTEFAP technique was used to determine the bacterial profile of each fermentation time at 24 and 48 h. Bacterial populations were identified at the species level. Species were grouped by substrate affinities (guilds) for cellulose, hemicellulose, pectin, starch, sugars, protein, lipids, and lactate. The 2 (co)products were a dried distillers grain (DDG) plus solubles produced from a low-heat drying process (BPX) and a high-protein DDG without solubles (HP). Chemical analysis revealed that BPX contained about 11.4% ether extract, whereas HP contained only 3.88%. Previous studies have indicated that processing methods, as well as fat content, of corn (co)products directly affect fermentation rate and substrate availability, but little information is available regarding changes in rumen bacterial populations. Fermentation profiles of intact and defatted BPX and HP were compared with alfalfa hay as a standard profile. Defatting before incubation had no effect on total gas production in BPX or HP, but reduced lag time and the fractional rate of fermentation of BPX by at least half, whereas there was no effect for HP. The HP feed supported a greater percentage of fibrolytic and proteolytic bacteria than did BPX. Defatting both DDG 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%). Information regarding the fermentation kinetics and bacterial population shifts when feeding corn (co)products may lead to more innovative processing methods that improve feed quality (e.g., deoiling) and consequently allow greater inclusion rates in dairy cow rations.
Key words: gas production, distillers grain, bacteria profile, pyrosequencing

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 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 fermenta-

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[1]Corresponding author: luis.tedeschi@tamu.edu
[2]Current address: ICM Inc., Colwich, KS 67030-0397.

tion 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 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 1. Chemical analysis of 3 intact feeds commonly fed in dairy cow rations

	Feed[1]		
Item	Alfalfa hay	BPX	HP
DM, % as fed	92.6	91.7	92.9
CP, % of DM	21.2	28.2	42.4
Soluble protein, % of CP	37.3	4.21	3.13
ADF protein, % of DM	1.30	1.44	2.65
NDF protein, % of DM	4.60	4.72	8.50
Estimated ruminal digestibility, % of 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, % of DM	26.3	27.6	22.9
Starch, % of DM	1.10	5.77	8.38
Sugar, % of DM	4.50	4.20	2.30
ADF, % of DM	36.3	7.92	10.1
NDF, % of DM	44.3	32.0	36.5
Lignin, % of DM	7.70	1.80	2.07
Fat, % of DM	2.00	11.4	3.88
Ash, % of DM	2.00	5.51	2.84

[1]Feeds analyzed: alfalfa hay = internal laboratory standard feed; BPX and HP-DDG = corn dried distillers grain (co)products where BPX undergoes a low-heat process before 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 version 9.2 (SAS Inst. Inc., Cary, NC) and R version 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]:

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

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

where Y is gas produced, mL; a represents the asymptote, mL; b represents the fractional degradation rate, h^{-1}; t is time, h; 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 were analyzed as a completely randomized design with factorial arrangements using 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 squares means were used for multiple comparisons using Tukey's adjustment for the P-value. A 3-way factorial arrangement of 2 feeds (HP or BPX) × 2 forms (intact or defatted) × 2 incubation times (24 or 48 h) was used (n = 16). The statistical model is shown in Eq. [3]. Only data applicable to all 3 blocks were used to analyze significant differences in pH between the 2 feeds in question:

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

where Y was the measured variable; Fd was HP or BPX; Fm was intact or 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 were used, only intact form data were included because alfalfa hay did not have a defatted form. In this case, a 2-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]:

$$Y_{ijk} = \mu + Fd_i + T_j + Fd \times T_{ij} + R_{k(ij)} + e_{ijk}, \quad [4]$$

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.

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 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 2.

RESULTS AND DISCUSSION

Gas Production Analysis

Table 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

Table 2. Prominent bacterial species identified by 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing grouped by their affinitive substrate guild

	Substrate guild[1]									
Species	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, FC = fiber carbohydrate, La = lactate.

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

Table 3. Comparison of the dynamics of the in vitro fermentation of alfalfa hay and 2 intact and defatted corn milling (co)products[1] using 2 nonlinear functions

		BPX		HP			
Item[2]	Alfalfa hay	Intact	Defatted	Intact	Defatted	SEM	*P*-value
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]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]BPX and HP = corn dried distillers grain (co)products where BPX undergoes a low-heat process and HP has high protein content.

[2]a represents the asymptote; b represents the fractional degradation rate; c represents lag time; d represents the asymptote of the second pool (assumed to be fiber); and e represents the fractional degradation rate of the second pool.

Table 4. Effects of feed, time, and form, and their interactions on pH and percentage of bacterial DNA recovered from mixed ruminal fluid fermented in vitro[1]

	Feed[2]		Time, h		Form[2]			*P*-value				
Item	BPX	HP	24	48	I	D	SEM	Feed	Time	Form	Fd × Fm[3]	T × Fm[3]
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[4]												
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
Hemicellulose, %	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, means 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.

[3]Fd × Fm = interaction of feed and form; T × Fm = interaction of time and form.

[4]Guild = bacteria combined into a substrate-utilizing group; given as percentage of all bacterial DNA recovered from each fermented sample; FC = fiber carbohydrate..

and fractional fermentation rate by at least half ($P < 0.02$).

pH Effects

Table 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 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

Table 5. Effects of the interaction of feed and time on the percentage of bacterial DNA recovered from mixed ruminal fluid fermented in vitro[1]

		Feed[3]							
		Alfalfa hay		BPX		HP			
Guild[2]	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, means 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]Guild = bacteria combined into a substrate-utilizing group; given as percentage of all bacterial DNA recovered from each fermented sample; FC = fiber carbohydrate.

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

[4]Main effects were different; see text.

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 5. 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 dif-

ferent (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.

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