Effects of including NaOH-treated corn straw as a substitute for wheat hay in the ration of lactating cows on performance, digestibility, and rumen microbial profile

E. Jami,*†¹ N. Shterzer,*¹ E. Yosef,* M. Nikbachat,* J. Miron,* and I. Mizrahi*²

*Department of Ruminant Sciences, Institute of Animal Science, Agricultural Research Organization, PO Box 6, Bet-Dagan, 50250, Israel

†Department of Molecular Microbiology and Biotechnology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat-Aviv, 69978, Israel

ABSTRACT

This study measured the effects of including 5% NaOH-treated corn straw (T-CS) as a substitute for 15% wheat hay in the control total mixed ration (TMR) of lactating cows on performance, digestibility, and rumen microbial profile. Two groups of 21 cows each, similar in initial performance, were fed individually 1 of the 2 TMR examined. Voluntary dry matter intake of cows fed the control TMR was 4.3% higher than that of the T-CS cows, but *in vivo* dry matter and organic matter digestibilities of both groups were similar. Crude protein digestibility was higher in the control cows but digestibility of neutral detergent fiber polysaccharides (cellulose and hemicelluloses) was higher in the T-CS TMR. This was followed by 4.6% reduction in rumination time of the T-CS group. A slightly higher milk yield was observed in the control cows compared with the T-CS group; however, milk fat and milk protein content were higher in cows fed the T-CS TMR. This was reflected in 1.3% increase in energy-corrected milk yield and 5.34% increase in production efficiency (energy-corrected milk yield/intake) of the T-CS cows compared with the control. Welfare of the cows, as assessed by length of daily recumbence time, was improved by feeding the T-CS TMR relative to the control group. As a whole, the rumen bacterial community was significantly modulated in the T-CS group in the experimental period compared with the preexperimental period, whereas the bacterial community of the control group remained unchanged during this period. Out of the 8 bacterial species that were quantified using real-time PCR, a notable decrease in cellulolytic bacteria was observed in the T-CS group, as well as an increase in lactic acid-utilizing bacteria. These results illustrate the effect of T-CS on the com-

position of rumen microbiota, which may play a role in improving the performance of the lactating cow.

Key words: corn straw, lactating cow, digestibility and performance, rumen microbiota

INTRODUCTION

As more land and resources are taken over for growing grains, less is available for forage crops and ruminants must make more use of field crop residues. The main field crop residues produced in the world by the grain manufacturers are corn, wheat, and rice straws, which contain about 70 to 80% cell wall constituents and represent a potential energy source for ruminants (Kossila, 1984). Availability of this energy to the animals is generally limited by the low voluntary intake, the chemical association between lignin and cell wall polysaccharides, and physical limitations of the cell wall components for microbial degradation (Theander and Aman, 1984; Miron and Ben-Ghedalia, 1987a,b). Chemical treatment is a processing method that breaks the bonds in a lignified matrix, thus liberating potentially digestible carbohydrates for rumen microbial attack. The effect on digestibility of various straws that results from NaOH treatment has been well documented (Jackson, 1977; Homb, 1984). Previous studies demonstrated that semi-dry alkali treatment of wheat straw disrupts ester linkages within the lignin-hemicellulose matrix and has a swelling effect on cellulose microfibrils (Miron and Ben-Ghedalia, 1987a,b; Yosef et al., 1994). The outcome is partial solubilization of the hemicellulose and labile phenolic fractions, resulting in an increase in voluntary intake and *in vitro* and *in vivo* DM digestibility (Miron and Ben-Ghedalia, 1987a,b). However, animal performance has not always been greatly increased when comparing untreated and NaOH-treated straws (Rexen and Knudsen, 1984; Wilkinson, 1984). To support optimal milk production throughout lactation, adequate amounts of energy must be supplied to the dairy cow and a minimal level of 17 to 21% roughage NDF is needed to prevent rumen

Received June 27, 2013.

Accepted November 16, 2013.

¹E. Jami and N. Shterzer contributed equally to this study.

²Corresponding author: itzhakm@agri.gov.il

acidosis and health problems (NRC, 2001). However, the availability of high-quality forages at economical prices can be limited during periods of poor growing conditions. For example, in many parts of the world, the use of chemically treated fibrous crop residues may be economical if the price of corn and corn silage is high. Low-quality forages and crop residues that have been treated with alkali have been fed successfully to beef cattle and sheep (Wilkinson, 1984). Additionally, Haddad et al. (1998) observed that the inclusion of up to 20% wheat straw treated with a combination of 3% NaOH and 3% Ca(OH)₂ in place of alfalfa in the diets of mid-lactation dairy cows had no negative effect on lactational performance.

Wheat silage and hay are the predominant forage sources fed to Israeli lactating cows. Untreated corn straw is produced in Israel and fed to heifers and dry cows but is not included in the TMR of lactating cows due to its low intake and digestibility. There is a lack of knowledge about the effect of including NaOH-treated corn straw (**T-CS**) as wheat hay replacer in the TMR of lactating cows on digestibility and milk performance. In addition, no data are available regarding the effects of feeding T-CS on the profile of rumen bacteria. The innovative genomic approach recently developed for quantitative and qualitative identification of rumen bacterial populations (Jami et al. 2012) might be used for the identification of changes occurring in the rumen bacterial population of lactating cows due to replacement of wheat hay by T-CS in the TMR.

The objective of this study was to measure the effects of including 5% NaOH-treated corn straw as a substitute for wheat hay in the TMR of lactating cows on milking performance, *in vivo* digestibility, and rumen microbial profile. Effects of NaOH treatment on corn straw composition and *in vitro* digestibility were also measured.

MATERIALS AND METHODS

NaOH Treatment, Diets, Cows, and Sampling Procedures

Corn straw (*Zea mays* commercial cultivar Pioneer P7532), including stems, leaves, and cob residues of corn plants after commercial combine harvest of the grains, was wilted in the field for a week, packed into 400-kg condensed bales and delivered to Beer-Tuvia Feeding Center (Be'er Tuvia, Israel). The bales were opened and the corn straw was chopped into 3- to 10-cm particles designated as untreated corn straw (**U-CS**). The U-CS (680 kg) was mixed for 15 min in a mixing wagon with 60 kg of concentrated NaOH solution (48% wt/wt) and 260 kg of water to create 1 t of 5% NaOH T-CS. The

wet T-CS was kept in a shaded area for 48 h before it was sampled for analyses and included in the TMR. The effect of NaOH treatment on the chemical composition and *in vitro* digestibility of corn straw is given in Supplementary Table S1 (<http://dx.doi.org/10.3168/jds.2013-7192>) together with wheat hay data. The T-CS was mixed in the experimental TMR at a level of 150 kg/t on a DM basis, as direct replacer for 15% wheat hay included in the control TMR. The compositions of the 2 TMR are in Supplementary Table S2 and their chemical compositions and *in vitro* digestibility are in Table 1. The replacement of 15% wheat hay in the control TMR by T-CS in the experimental TMR dictated some necessary changes in protein sources to obtain similar CP levels (16.5%) in both TMR. In addition, the unnecessary buffer NaHCO₃ was removed from the T-CS TMR due to NaOH addition. These changes reduced the price of the experimental TMR by \$0.025 per kg of DM (Table 1).

The experimental and control TMR were freshly prepared every day, delivered to the Agricultural Research Organization individual feeding dairy barn in Bet-Dagan (Israel), and fed *ad libitum* to 1 of the 2 groups of lactating cows. The 2 groups were similar in lactation number (3.3) and initial DIM and milk production. Cows were blocked by DIM (60 to 120 vs. 120 to 180) and daily milk production (30 to 36 vs. 36 to 42 kg/cow) and randomly assigned within block to pairs fed the 2 dietary treatments. Two weeks before startup of the experiment, both groups were fed the control TMR and sampled for rumen fluid and bacterial population analyses. The 2 groups were then fed the 2 TMR (control vs. experimental) for 2 wk of adaptation to the TMR, followed by 6 wk of experimental period. The TMR were fed once daily at 1000 h *ad libitum*, allowing for 5 to 10% refusals, and cows were milked 3 times daily at 0600, 1400, and 2200 h. Cows were fed individually via a computerized monitoring system designed to electronically identify individual cows and to automatically control and record each cow's daily feed intake (Miron et al., 2003). Daily DMI of individual cows was determined based on DM content in TMR sampled daily and feed refusals. Recumbence time for each cow, an indicator of animal welfare (Drissler et al., 2005), was recorded daily by an automatic meter located within each cow's pedometer (Afimilk Ltd., Kibbutz Afikim, Israel), and BW data were recorded by an automatic walk-over scale 3 times per day while cows were entering the milking parlor. Changes in BW were calculated as the gap between the week before the onset of the experiment and the last week of the experiment.

Milk yield (kg) was recorded daily for each cow by an automatic meter (Afimilk Ltd.). Milk samples were collected in 3 sequential milkings on a weekly basis

Table 1. Chemical composition (g/kg of DM; $\pm$ SE) and in vitro digestibility (%) of the control TMR and of the experimental TMR containing NaOH-treated corn straw¹ as substitute for wheat hay fed to lactating cows

Item	TMR	
	Control	Experiment
DM (g/kg of TMR)	653 $\pm$ 18.9	613 $\pm$ 15.1
OM	919 $\pm$ 17.5	886 $\pm$ 14.6
CP	165 $\pm$ 3.3	165 $\pm$ 3.2
Ether extract	48.7 $\pm$ 0.96	48.4 $\pm$ 0.98
NDF	381 $\pm$ 7.1	406 $\pm$ 14.6
Hemicellulose	199 $\pm$ 4.1	211 $\pm$ 6.4
Cellulose	136 $\pm$ 9.3	139 $\pm$ 4.8
Lignin	44.7 $\pm$ 7.03	42.0 $\pm$ 1.08
Forage NDF ²	158 $\pm$ 7.2	170 $\pm$ 5.2
In vitro DM digestibility (%)	74.4 $\pm$ 0.18	72.1 $\pm$ 0.19
Daily TMR price (\$/kg of DM)	0.39	0.37

¹The NaOH treatment was conducted by mixing for 15 min in a mixing wagon 4,750 kg of chopped corn straw DM + 2,250 kg of NaOH-water solution containing 20% (wt/wt) NaOH.

²Forage NDF is based on analysis of NDF content of the wheat silage and wheat hay in the control TMR versus wheat silage and NaOH-treated corn straw in the experimental TMR.

throughout the study. Milk samples were stored at 4°C in the presence of 2-bromo-2-nitropropane-1,3-diol, until infrared analysis for fat, true protein, and lactose content, using a MilkoScan 4000 analyzer (Foss Electric A/S, Hillerød, Denmark). Energy-corrected milk yield was calculated using the following equation (NRC, 2001): ECM (kg/d) = milk yield (kg/d) $\times$ {[0.3887 $\times$ milk fat (%)] + [0.2356 $\times$ milk protein-urea (%)] + [0.1653 $\times$ milk lactose (%)]}/3.1338 MJ/kg.

The animal performance study was carried out according to the guidelines and under the supervision of the Agricultural Research Organization Animal Care Committee.

Chemical Composition and In Vitro Digestibility Analyses

Samples of the experimental and control TMR as well as fecal samples from the experiment were dried for 48 h in an aerated 60°C oven, ground to pass a 1-mm sieve, and analyzed according to the methods of the AOAC (1990) for DM (method no. 925.40), total N (method no. 984.13), ether extract (method no. 920.39), and ash (method no. 923.03). Crude protein was calculated as N $\times$ 6.25. Determination of NDF was as described by Van Soest et al. (1991) using heat-stable amylase without sodium sulfite. Content of ADF, ADL, and residual ash were determined using the sequential method of Van Soest et al. (1991). An Ankom Fiber Analyzer (Ankom₂₂₀; Ankom Technology Corp., Macedon, NY) was used to extract and filter NDF and ADF. Hemicellulose was calculated as NDF – ADF, cellulose as ADF – ADL, and lignin as ADL – residual ash.

In vitro DM digestibility of the U-CS, T-CS, wheat hay, and the 2 TMR was determined according to the

2-stage technique of Tilley and Terry (1963), using rumen fluid taken from the rumen of 2 cows equipped with a rumen fistula, fed TMR containing 150 g of T-CS + 150 g of wheat hay + 300 g of wheat silage + 400 g of concentrate mixture per kilogram of TMR DM.

In Vivo Digestibility Measurements

At wk 5 of the experiment, 4 d were assigned for daily sampling of TMR, feed refusals, and feces from 10 cows involved in each of the 2 treatments. Cows for the digestion study were selected in pairs to obtain 2 groups from the 2 dietary treatments similar in intake and milk yield. Estimation of daily fecal excretion was based on indigestible NDF concentrations in feces and the TMR. Samples of the TMR and refusals were composited on a daily basis, dried (60°C for 48 h), and ground through a 1-mm sieve (S-M-100; Retsch GmbH, Haan, Germany). Fecal grab samples were collected 3 times daily for 4 d, at 8-h intervals, each day 2.5 h later than the preceding day. Fecal samples were composited (on a DM basis) from each cow during the collection period, dried at 60°C for 48 h in a forced-air oven, ground to pass through 1-mm sieve, and then used for chemical analyses as described above and for indigestible NDF content in the TMR and fecal pooled samples. The 2-stage in vitro digestibility technique of Tilley and Terry (1963) was used to analyze the indigestible NDF content (after incubation with rumen fluid for 72 h) of the TMR and of pooled fecal samples of each cow for in vivo digestibility calculation according to Adin et al. (2009). The indigestible NDF was used as an internal marker for the apparent total-tract DM digestibility analysis. The in vivo digestibility of each chemical component (i.e., DM,

CP, NDF, cellulose, and hemicellulose) was calculated in each cow using the average individual DMI and fecal output.

Rumen pH Measurement

Once per week, 500 mL of rumen fluid samples free of saliva was collected with a rumen vacuum sampler from each cow 1 h before feeding (0900 h) and 3 h after feeding (1300 h). The pH values were immediately determined by a portable pH meter.

Pattern of Rumination

All cows were equipped with rumination tag monitoring systems that measured the duration of daily rumination (min/d) over the 6 wk of the experiment. Commercial tags (Hi-Tag TM; SCR Engineers Ltd., Netanya, Israel; <http://www.scr.co.il>), which are routinely used in Israeli herds, were used for this measurement. The rumination data was automatically downloaded after each milking, stored in the herd computer, and analyzed by special software (SCR Engineers Ltd., Netanya, Israel). The basic principle of the rumination time measurement is to analyze the isolated chewing sounds during rumination, excluding the sounds related to eating. Data was stored in “2-h cells,” at 2-min resolution.

Rumen Bacterial Isolation

Rumen digesta was sampled from the experimental group ($n = 5$) twice, once before the beginning of the differential experiment and once 1 mo after the beginning of the experiment. The control group ($n = 5$) fed wheat hay containing TMR was also sampled for comparison. Rumen digesta was sampled 6 h after feeding, as described above. Detachment of the fiber-adherent microbial populations and isolation of both the fiber-adherent and planktonic populations was performed as described by Stevenson and Weimer (2007). Briefly, total rumen digesta from each cow was homogenized by blender for 2 min, the homogenate was centrifuged at $10,000 \times g$ for 30 min at 4°C, and the pellet was dissolved in extraction buffer [100 mM Tris-HCl, 10 mM EDTA, and 150 mM NaCl (pH 8.0)]; 1 g of pellet was dissolved in 4 mL of buffer and incubated at 4°C for 1 h, as chilling has been shown to maximize the release of particle-associated bacteria from ruminal contents. The suspension was then centrifuged gently at $500 \times g$ for 15 min at 4°C to remove ruptured plant particles while keeping the bacterial cells in suspension. The supernatant was then passed through 4 layers of cheesecloth and centrifuged ($10,000 \times g$ for 25 min at 4°C), and

the microbial pellets were kept at -20°C until DNA extraction.

DNA Extraction

The DNA extraction was performed as described by Stevenson and Weimer (2007). Cell lysis was achieved by bead disruption with phenol, followed by phenol/chloroform DNA extraction. The final supernatant was precipitated with 0.6 volume of isopropanol and resuspended overnight in 50 to 100 μL of TE buffer [100 mM TRIS (pH 8.0) and 1 mM EDTA], and then stored at 4°C for short-term use, or archived at -80°C .

Automated Ribosomal Intergenic Spacer Analysis

This method uses the natural variation in length occurring between the 16S and 23S of different bacterial taxa to generate a whole bacterial community fingerprint, which can be further analyzed using statistical evaluation of the changes between the 2 time points. The DNA from all rumen samples was subjected to PCR amplification for automated ribosomal intergenic spacer analysis (**ARISA**; Fisher and Triplett, 1999). Two technical replicates were performed for each DNA sample. The oligonucleotide primers ITSf (5'-GTCTG-TAACAAGGTAGCCGTA-3') and ITSrtet (5'-GC-CAAGGCATCCAAC-3') used were as described by Welkie et al. (2010) for ARISA of rumen bacteria, with the fluorescent molecule being tetrachlorofluorescein (TET). The ARISA PCR were carried out in 15- μL volumes containing Fermentas DreamTaq Master Mix (Fermentas, Madison, WI), 0.5 μL of 10 μM stock solution for each primer, 20 ng of template DNA, and 4.5 μL of nuclease-free water. The PCR were carried out using a SensoQuest thermocycler (SensoQuest GmbH, Göttingen, Germany) under the following conditions: 94°C for 2 min (1 cycle), followed by 30 cycles of 94°C for 1 min, 55°C for 60 s, and 72°C for 120 s, and finally 1 cycle of 72°C for 5 min.

ARISA Resolution and Analysis

The PCR products were analyzed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems Inc., Carlsbad, CA). The labeled fragments were separated on the capillary sequencer along with a custom-made ROX-labeled 250- to 1,150-bp standard (BioVentures Inc., Murfreesboro, TN). Raw data generated by the genetic analyzer were initially analyzed using GeneMarker software (SoftGenetics LLC, State College, PA) according to Kovacs et al. (2010). After performing accurate size calling using the program, all data were exported to Excel (Microsoft Corp., Redmond,

WA) for further analysis. All operational taxonomic units (OTU) with fluorescence intensity of 10 relative fluorescence units (RFU) or lower were excluded. The remaining OTU were binned as described by Brown et al. (2005), with the following parameters: bins of 3 bp (± 1 bp) for fragments up to 700 bp in length, bins of 5 bp for fragments between 700 and 1,000 bp in length, and bins of 10 bp for fragments above 1,000 bp. Intensities were then summed for each bin. Next, relative intensities for each binned OTU in a given sample were calculated and OTU that constituted less than 0.1% of the total intensity of the sample were excluded. Technical duplicates were compared as if they were individual samples and served as an internal control for the quality of the analysis. Duplicates that were not similar to each other were further checked for technical run problems and were either discarded or run again. The file generated by Excel was transferred to PAleontological STatistics (PAST) software (Hammer et al., 2001; <http://folk.uio.no/ohammer/past/>). The Bray-Curtis distance indexes, as well as the nonmetric multidimensional plotting, were performed using this program. The data were further processed using the Microsoft Excel program.

Real-Time PCR

Quantitative real-time PCR analysis was performed to investigate the relative abundance of 8 bacterial species, 1 genus, and total Archaea through amplification of their copy of the 16S rRNA gene, using the primers shown in Supplementary Table S3 (<http://dx.doi.org/10.3168/jds.2013-7192>; Walter et al., 2000; Stevenson and Weimer, 2007; Zhou et al., 2009). Standard curves were generated for each bacterial strain selected by amplifying a serial 2-fold dilution of gel-extracted PCR products obtained by the amplification of each amplicon. A standard curve was also generated for the total bacterial 16S rRNA gene in the samples by amplifying 10-fold dilutions of the gel-purified PCR product of 1 rumen sample. The standard curves were obtained using 4 dilution points, and were calculated using Rotor-Gene 6000 series software (Qiagen GmbH, Hilden, Germany). Subsequent quantifications were calculated with the same program using the standard curve generated in each run (equating to 1 bacterial species), and at least 1 known purified product dilution used for the standard curves was added to each quantification reaction to assess the reproducibility of the reactions. All obtained standard curves met the required standards of efficiency ($R^2 > 0.99$; $90\% < E < 115\%$, where E = efficiency). Real-time PCR was performed for each DNA sample in triplicate, in a 10- μ L reaction mixture containing 5 μ L of Absolute Blue SYBR Green Master

Mix (Thermo Scientific, Waltham, MA), 0.5 μ L of each primer (10 μ M working concentration), 3 μ L of nuclease-free water, and 2 μ L of 10 ng/ μ L DNA template. Amplification involved 1 hold cycle at 95°C for 15 min for initial denaturation and activation of the hot-start polymerase system, and then 40 cycles at 95°C for 10 s, followed by annealing for 15 s at 60°C and extension at 72°C for 20 s. To determine the specificity of amplification, a melting curve of PCR products was monitored by slow heating with an increment of 1°C and holding for 10 s from 60 to 99°C, with fluorescence collection at 1°C intervals. Quantification of the selected taxa was performed by dividing the number of the specific bacterial count obtained for each bacterium, using the appropriate set of primers, to the total bacterial count obtained by the amplification of the universal bacterial primers.

Statistical Analyses

Differences between the 2 types of TMR in Table 2 with respect to in vivo digestibility were tested for significance by F-test of ANOVA using JMP-5 software (SAS Institute, 1996). The Tukey test (SAS Institute, 1996) was used for comparison among means.

Daily feed intake (Table 2), BW, milk yield, milk composition and milk component yields, ECM yield, rumination, and recumbence time (Table 3) were averaged by cow for the 42 d in the experiment (42 repeated measures for each cow). Data were analyzed as a randomized complete block design using the mixed model of SAS software (SAS Institute, 1996). The statistical model included the effects of dietary treatment, block, and cow by block interaction. Cow within block was delineated as the random effect in the model and used to test treatment and block effects, whereas repeated measurement of daily data were tested for the residual error.

The rumen pH and in vivo digestibility data (10 cows = replicates per each dietary treatment) were analyzed for significance by F-test of ANOVA using JMP-5 software (SAS Institute, 1996). The Tukey test (SAS Institute, 1996) was used for comparison among means.

RESULTS

Effect of NaOH Treatment on Corn Straw

Data in Supplementary Table S1 (<http://dx.doi.org/10.3168/jds.2013-7192>) show that the semi-dry treatment of corn straw with 5% NaOH reduced NDF content by 14%, while solubilizing 35% of the hemicelluloses, 8.7% of the cellulose, and 11.3% of the lignin fractions. These changes resulted in a 9.1% increase in

Table 2. Intake and in vivo digestibility of dietary components in mid-lactation cows individually fed for 6 wk the control TMR containing 15% wheat hay versus the experimental TMR containing NaOH-treated corn straw (T-CS)¹ as a substitute for wheat hay

Item	TMR		SEM	P-value
	Control	T-CS		
No. of cows	21	21		
DMI (kg/cow per day)	29.0 ^a	27.8 ^b	0.10	0.01
DM digestibility (%)	67.1	65.9	0.38	0.097
OM digestibility (%)	69.0	70.0	0.38	0.20
CP digestibility (%)	70.4 ^a	65.9 ^b	1.15	0.044
NDF digestibility (%)	47.1	49.6	0.74	0.094
Cellulose digestibility (%)	47.0	50.9	1.13	0.078
Hemicellulose digestibility (%)	51.3 ^b	57.6 ^a	1.18	0.001
Rumination (min/cow per day)	439 ^a	419 ^b	2.37	0.012
Rumen pH	6.32 ^b	6.48 ^a	0.03	0.05

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

¹The NaOH treatment was conducted by mixing for 15 min in a mixing wagon 4,750 kg of chopped corn straw DM + 2,250 kg of NaOH-water solution containing 20% (wt/wt) NaOH.

in vitro DM digestibility of the corn straw. Untreated corn straw cannot be included in high quantities in lactating cow rations (Oji et al. 1977; Wilkinson, 1984). But, because in vitro DM digestibility of the T-CS was similar to that of wheat hay (62.1 and 63.6%, respectively), it motivated the use of T-CS as wheat hay replacer in TMR fed to lactating cows. The control TMR contained 3.7% more OM, 6.2% less NDF, 7.1% less roughage NDF, and similar protein and fat content to that of the T-CS TMR. In vitro DM digestibility was 3.2% higher in the control TMR than the T-CS TMR (Table 1).

Performance, Intake, and Digestibility

Voluntary DMI and in vivo digestibility data of the 2 TMR individually fed to lactating cows are in Table 2. Cows fed the control TMR ingested 4.3% more DM

than the T-CS cows, but the in vivo DM and OM digestibility of both groups were similar. Crude protein digestibility was higher in the control cows but digestibility of NDF and NDF polysaccharides (cellulose and hemicelluloses) tended to be higher in the T-CS TMR ($P < 0.1$). Although the control and T-CS groups ingested similar quantities of NDF (11.05 and 11.29 kg/cow per day, respectively) the slight advantage of the T-CS group with respect to NDF polysaccharide digestibility was followed by 4.6% reduction in rumination time of the cows and slightly higher rumen pH values (Table 2). The in vitro digestibility was not statistically different between the 2 groups, but was higher than the in vivo digestibility.

The lactation performance data of cows fed the 2 dietary treatments are in Table 3. The higher intake of digestible OM in the control cows resulted in slightly higher milk yield ($P < 0.06$) of this group compared

Table 3. Lactational performance of mid-lactation cows individually fed for 6 wk the control TMR containing 15% wheat hay versus the experimental TMR containing NaOH-treated corn straw (T-CS)¹ as a substitute for wheat hay

Item	TMR		SEM	P-value
	Control	T-CS		
No. of cows	21	21		
Milk yield (kg/cow per day)	44.4	43.9	0.16	0.06
Milk fat (%)	3.06 ^b	3.21 ^a	0.015	0.01
Milk true protein (%)	3.03 ^b	3.07 ^a	0.007	0.01
Milk lactose (%)	4.70	4.69	0.220	0.16
ECM yield (kg/cow per day)	38.0 ^b	38.5 ^a	0.14	0.05
Production efficiency (ECM yield/DMI)	1.31 ^b	1.38 ^a	0.005	0.02
BW change (kg/cow per day)	0.26	0.14	0.05	0.15
Recumbence (min/cow per day)	542 ^b	586 ^a	3.22	0.01

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

¹The NaOH treatment was conducted by mixing for 15 min in a mixing wagon 4,750 kg of chopped corn straw DM + 2,250 kg of NaOH-water solution containing 20% (wt/wt) NaOH.

with T-CS-fed cows. However, milk fat and milk protein content were higher in cows fed the T-CS. This was reflected in a 1.3% increase in ECM yield of the T-CS cows. The lower intake and higher ECM yield of the T-CS cows were reflected in a 5.34% increase in ECM production efficiency compared with the control cows. The welfare of the cows as assessed by length of daily recumbence time was improved by 44 min in the T-CS cows compared with the control group.

Whole Bacterial Community Analysis

Whole bacterial community analysis was performed using ARISA to compare the animals of each group before the experiment, when fed the control TMR, and during the experiment, when fed the 2 different TMR. Analysis of similarities revealed that the switch in diet from the control wheat hay TMR to the T-CS TMR resulted in a significant modulation of the rumen bacterial community ($P < 0.05$; Figure 1). Analysis of similarities statistically evaluates the degree of similarity between groups; R-values are a measure of the degree of difference between 2 groups, where values closer to 1 indicate less similar groups. Whereas the control group showed no differences between the 2 time points, the experiment group exhibited 2 significantly different bacterial populations (Figure 1).

Bacterial and Archaeal Composition Analysis

Following the ARISA results, we used real-time PCR to quantify bacterial species that are thought to be the primary degraders of cell wall components, as well as 2 other soluble sugar utilizers. These bacteria included the 3 main cellulolytic bacterial species, 3 *Prevotella* species that are considered to be hemicellulose degraders, and the overall *Prevotella* genus. In addition, we quantified *Selenomonas ruminantium* which is considered to utilize soluble sugars as well as lactate, and *Succinivibrio dextrinosolvens*, which is a soluble sugar utilizer. This analysis revealed significantly different abundance values in 4 of the species tested ($P < 0.05$) between the 2 different diets (Figure 2A; Supplementary Table S4, <http://dx.doi.org/10.3168/jds.2013-7192>). The abundance of the functionally diverse *S. ruminantium* increased by 2-fold. A 2-fold decrease in abundance was observed in 2 of the 3 main cellulolytic bacterial species in the rumen: *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* (using a *t*-test; $P < 0.05$). A 1.5-fold increase was observed in the abundance of *Ruminococcus albus*, the third cellulolytic species, but this change was not statistically significant. Although the abundance of the *Prevotella* genus did not show a significant change, the composi-

Figure 1. Automated ribosomal intergenic spacer analysis similarity distance of the bacterial communities of the experimental group (A) and the control group (B) in the preexperimental and experimental periods. Control group cows were fed control TMR in both preexperimental and experimental periods; experimental group cows were fed control TMR during the preexperimental period and treated corn straw (T-CS) TMR during the experimental period. The distance between the samples was based on the similarity in operational taxonomic unit (OTU) composition of each sample, calculated by the Bray-Curtis similarity index and plotted using nonmetric multidimensional scaling. Each point represents a different sample plotted on a 2-dimensional graph according to its OTU composition and abundance. A greater distance between 2 points implies a lower similarity between them, whereas samples with a more similar bacterial composition and abundance are clustered closer together. Analyses of similarity R-values, indicating the degree of difference between the groups, are displayed in the lower right corner of the graph along with their corresponding P-values.

tion of the species within this genus was altered under the different feeding regimens. This differential effect within the *Prevotella* genus could be observed with the 2-fold increase in *Prevotella brevis* and a 6-fold decrease in *Prevotella bryantii* (Figure 2B; Supplementary Table S4, <http://dx.doi.org/10.3168/jds.2013-7192>). The archaeal communities in the 2 different diets were not statistically different.

No statistical differences were observed between the 2 time points in the control group, which continuously received the same wheat hay TMR throughout the time of the experiment, reducing the potential effect of time and environmental changes on the alteration in bacterial composition seen in the T-CS group (data not shown).

DISCUSSION

Effects of T-CS on Intake, Digestibility, and Performance

Our aim in this study was to evaluate the treatment of corn straw with NaOH as a potential inexpensive alternative for wheat hay in the TMR of dairy cows during lactation. Although corn for grain and straw is one of the main crops grown in Israel, the low intake and digestibility of corn straw limits its utilization in cows during lactation. Treatment of various straws with NaOH and the resulting beneficial effect is well documented (Homb, 1984; Rexen and Knudsen, 1984; Jackson, 1977). In those studies, NaOH treatment of various types of straw invariably improved the digestibility of the feed. The improvement is thought to stem from the disruption of ester linkage of lignin and hemicelluloses, making carbohydrates more available for bacterial enzymatic digestion (Miron and Ben-Ghedalia, 1987a,b; Yosef et al., 1994). In the current study, the in vitro DM digestibility of the T-CS TMR (72.1%; Table 1) was 9.4% higher than the in vivo DM digestibility of this diet in cows (Table 2), and a similar difference of 10.9% was observed between in vitro and in vivo DM digestibility values of the control TMR. This difference could be attributed to the effects of high intake ($\times 4$ maintenance level) that increases the rate of particulate passage and decreases the rate of ruminal fiber digestion (Wilkinson, 1984; NRC, 2001).

In the present study, we showed that cows fed T-CS had significantly better feed utilization efficiency for ECM production than cows fed wheat hay (Table 3). Welfare parameters such as recumbence time were also improved in these cows. Voluntary DMI was 4.1% lower in the cows fed T-CS than in the control group (Table 2), possibly attributed to the higher NDF and roughage NDF content of the T-CS TMR (Table 1). The higher

Figure 2. Quantification of known rumen microbial taxa in the samples using real-time PCR. Each graph displays the log ratio between selected taxa and total bacteria in samples of the experimental group in the preexperimental and experimental periods. Experimental group cows were fed control TMR during the preexperimental period and treated corn straw (T-CS) TMR during the experimental period. The y-axis denotes the log ratio between the taxa quantified and total bacteria and the x-axis denotes the taxa quantified. Whiskers represent SEM and asterisks (*) denote significant differences of abundances between the different diets ($P < 0.05$). (A) Total Archaea and different bacterial species. (B) Total *Prevotella* genus and different *Prevotella* species.

ECM yield of the T-CS cows resulted from higher fat and protein content in their milk (Table 3). This could be a result of higher digestibility of NDF and NDF polysaccharides in the T-CS cows, while having a similar NDF intake compared with the control group: 11.3 and 11.1 kg/d, respectively. Both daily recumbence time and daily rumination time were suggested in the literature as parameters for estimating cow health and welfare (Drissler et al., 2005; Jami et al. 2012). In the current study, cows fed the T-CS TMR had significantly shorter rumination time and longer recumbence time than the control cows. Shorter rumination time could be explained by the sensitivity of rumination time to ruminal parameters, such as the increased NDF digestibility seen in the T-CS group. Recumbence time is known as a direct indicator of cow comfort (Drissler et al., 2005), possibly associated with higher production, reflected in this study by the higher ECM yield of the T-CS cows.

Effects of T-CS Diet on the Rumen Bacterial and Archaeal Community

This study showed that the inclusion of T-CS in the TMR as a substitute for wheat hay affected the composition of the overall ruminal bacteria in lactating cows, as reflected by the ARISA and analysis of similarities results (Figure 1A). Nevertheless, this effect did not influence all examined microorganisms in the same manner, with some increasing and others decreasing in abundance. No observable changes occurred in bacterial composition within the control group receiving the wheat hay TMR throughout the experiment (Figure 1B). This finding is with concurrence with previous reports showing high conservation of the bacterial community when the animals are kept on the same diet (Jami et al. 2012). This degree of stability within the rumen bacterial community was also observed in another study showing little difference in composition within a specific cow at different sampling times (Li et al., 2009).

A significant decrease in abundance was observed in 2 of the main known cellulolytic bacterial species (Figure 2A; Supplementary Table S4, <http://dx.doi.org/10.3168/jds.2013-7192>). This could stem from the existence of other uncultured cellulolytic bacteria that could potentially occupy this niche when the abundance of *F. succinogenes* and *R. flavefaciens* decreased. Similar trends were recently observed in another work in which cellulolytic bacteria count decreased, even though cellulose degradation did not decrease and even increased (Jami et al. 2012). In the present study, no decrease was observed in cellulose content and cellulose in vivo digestibility between the T-CS TMR group and

the control TMR (Tables 1 and 2), and we even noted an increase in both of these parameters. We suggest that because NaOH treatment causes cellulose fibrils to swell, cellulose fibers may become more susceptible to degradation by other, unknown bacteria with cellulolytic potential, as it has been established that cultured bacteria represent only a small fraction of the overall bacterial species in the rumen (Stevenson and Weimer, 2007). Thus, the existence of other cellulolytic bacteria that occupy a slightly different niche and are more suited for degradation of NaOH-treated cellulose might explain the lack of decrease in overall cellulose and NDF degradation and the increase in hemicellulose in vivo digestion.

Even though no observable increase occurred in the *Prevotella* genus abundance, a notable shift was observed in several of the species quantified from this genus (Figure 2B; Supplementary Table S4, <http://dx.doi.org/10.3168/jds.2013-7192>). *Prevotella* is the most abundant genus in the rumen, accounting for over 70% of the total bacterial taxa in the rumen in some cases (Stevenson and Weimer, 2007; Jami and Mizrahi, 2012; Jami et al., 2013). The shift in this very abundant genus may be a good indicator of changes in environmental conditions in the rumen, as it was established that this genus has a wide variety of niches that are specific to individual species within the genus (Avguštin et al., 1997; Ramsak et al., 2000; Purushe et al., 2010). A previous comparative genome analysis of *Prevotella ruminicola* and *P. bryantii* revealed that these 2 species have different glycan degradation capabilities, with *P. ruminicola* having a higher presence of genes encoding glycan-degrading enzymes and *P. bryantii* having more extended pectin-cleaving abilities with a significantly larger number of pectate lyases, polygalacturonases, pectin methylesterases, and α -rhamnosidases (Purushe et al., 2010). These different genomic attributes may explain the different response of these species to the NaOH treatment, as it alters the cell wall structures, thereby potentially creating an advantage or disadvantage for the different species.

Among lactate and soluble sugar utilizers, *S. ruminantium* show a significant increase in the T-CS group (Figure 2A; Supplementary Table S4, <http://dx.doi.org/10.3168/jds.2013-7192>). This may be the result of the solubilization of plant cell wall components such as cellulose and hemicellulose in response to the NaOH treatment (Supplementary Table S1, <http://dx.doi.org/10.3168/jds.2013-7192>), which could have resulted in a higher content of soluble carbohydrates in the T-CS. The augmentation in soluble sugars, which are substrates for *S. ruminantium*, might explain this increase.

CONCLUSIONS

The switch from wheat hay TMR to T-CS TMR had a significant effect on the bacterial community within the rumen, which was also accompanied by a beneficial effect on rumen pH and digestibility of NDF and NDF polysaccharides. This was reflected in similar NDF intake of the 2 groups, but higher milk fat and milk protein content, increase of 1.3% in ECM yield, and 5.34% in the production efficiency (ECM yield/intake) of cows fed the T-CS TMR compared with the control group. The mechanism by which the effect of T-CS is carried out in modifying the ruminal bacterial population warrants further research. Understanding these forces that influence rumen microbial populations may contribute to future development of rumen microbial modulations toward improvement of cow performance.

ACKNOWLEDGMENTS

We express our appreciation to the Agricultural Research Organization (ARO, Bet-Dagan, Israel) dairy farm staff and the staff of Darom dairy farm (Kibbutz Gat, Israel) for their help, and to the Massuot Itzhak Feeding Center (Massuot Itzhak, Israel). This study was supported by the Israel Dairy Board Foundation (Yehud, Israel) Projects no. 362-0296 and 362-0316.

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