EFECTO DE DIFERENTES POLISACÁRIDOS SOLUBLES NEUTRO-DETERGENTE EN LA CINÉTICA DE DIGESTIÓN *IN VITRO* DE LA FIBRA NEUTRO-DETERGENTE FORRAJERA Y EN LA SÍNTESIS DE PROTEÍNA MICROBIANA

The Effect of Different Neutral-Detergent-Soluble Polysaccharides in Digestive Cynetics *In Vitro* of Neutral Detergent Forrage Fiber and the Synthesis of Microbial Protein

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ABSTRACT

The purposes of this experiment were to compare the pH-independent effects of starch, fructans, and pectins, described in this study as neutral detergent-soluble polysaccharides (NDSP), on forage neutral detergent fiber (NDF) digestion kinetics and microbial protein synthesis using the in vitro ruminant digestion technique. Isolated NDF from alfalfa (A), Bermuda grass (B), and timothy (T) was fermented alone (C) or with dahlia tuber inulin (I), citrus pectin (P), or corn starch (S) as NDSP sources. In blended samples, the NDF:NDSP ratio was 60:40. Individual forages (A, B or T) were fermented in separate duplicate runs using 0, 2, 4, 6, 12, 18, 24, 30, 36, 48, 72, and 96 h time points. Trichloroacetic acid was used to precipitate microbial and substrate crude protein in triplicate treatments and blanks at 0 and 24 h. The 24 h treatment values corrected for blank and 0 h treatment means estimated microbial crude protein yield (TCAP) at 24 h. Kinetics of NDF digestion were calculated by fitting the NDF disappearance data to the Mertens model. Both fermentation lag time (h) and the fractional rate of digestion (Kd; h^{-1}) were calculated iteratively by NLIN's Marquardt procedure of SAS. Results are listed in treatment order C, I, P, and S. The NDF kd values were 0.084, 0.099, 0.049, and 0.139 for A, 0.040, 0.035, 0.026, and 0.062 for B, and 0.069, 0.077, 0.069, and 0.088 for T. Lag time (hours) values were A: 6.73, 7.72, 0.07, and 8.03; B: 7.02, 7.64, 8.86, and 10.27; and T: 6.80, 6.41, 3.71, and 7.23. The ΔTCAP values (mg of CP/g of substrate OM) were A: 32.86, 75.95, 50.62, and 59.29; B: 29.26, 53.75, 24.70, and 51.24; and T: 40.48, 79.77, 51.30, and 68.81. Neither the lag time nor Kd were affected by NDSP addition to forage NDF (P>0.05). The ΔTCAP of forage incubated with P was always less than that incubated with I and S, and the effect of both I and S were similar except for Alfalfa NDF. The neutral detergent-soluble polysaccharides did not affect kinetics of forage NDF digestion when pH of cultures was maintained over 6.6 but, they differed in their effects on microbial protein production in vitro. Overall, treatment I and S favored greater in vitro microbial protein production than P.

Key words: Digestion kinetics, fiber, polysaccharides, microbial protein.

RESUMEN

Los objetivos de este experimento fueron comparar los efectos del almidón, fructosanos y pectinas, descritos en este estudio como polisacáridos solubles neutro-detergente (PSND) en la cinética de digestión de la fibra neutro-detergente (FND) forrajera y en la síntesis de proteína microbiana, independientemente del pH, a través de la técnica de digestión ruminal *in vitro*. Se fermento fibra neutro-detergente (FND) extraída de alfalfa (A), bermuda (B) y timoty (T), bien sola (C) o mezclada con inulina proveniente del tubérculo dalia (I), pectina de cítricos (P) o almidón de maíz (S) como fuentes de PSND. La proporción de FND: PSND en las muestras de las diferentes mezclas fue 60:40. Cada forraje (A, B o T) fue incubado en tandas separadas y en replicado durante 0, 2, 4, 6, 12, 18, 24, 30, 36, 48, 72, y 96 hrs. Se uso ácido tricloroacético para precipitar la proteína microbiana y la proteína cruda proveniente del substrato (ΔPTCA) en los tratamientos por triplicado y en los blancos a las horas 0 y 24. La producción de proteína cruda micro-

Recibido: 10 / 05 / 2002. Aceptado: 06 / 11 / 2003.

biana (PATC) a las 24 hrs fue estimada utilizando los valores de PTCA corregidos, a través de los blancos, para cada tratamiento a las 24 hrs y los promedios de PTCA corregidos a la hora 0. La cinética de digestión de FND fue calculada fijando los datos de desaparición de FND al modelo de Mertens. El lag (h) y la velocidad de digestión fraccional (Kd; h^{-1}) fueron calculadas iterativamente mediante regresión no lineal en SAS, utilizando el método de Marquardt. Los resultados por tratamiento son listados en el orden C, I, P, y S. Los valores de Kd de FND fueron 0,084; 0,099; 0,049 y 0,139 para A; 0,040; 0,035; 0,026 y 0,062 para B; y 0,069; 0,077; 0,069 y 0,088 para T. Los valores de lag (horas) fueron 6,73; 7,72; 0,07 y 8,03 para A; 7,02; 7,64; 8,86 y 10,27 para B; y 6,80; 6,41, 3,71 y 7,23 para T. Los valores de PATC (mg/g de materia orgánica del substrato) fueron 32,86; 75,95; 50,62 y 59,29 para A; 29,26; 53,75; 24,70 y 51,24 para B y 40,48; 79,77; 51,30 y 68,81 para T. Los polisacáridos solubles neutro-detergente añadidos a la FND no afectaron el lag ni tampoco Kd de manera significativa (P>0,05). Los valores de ∆PATC para las FND forrajeras que fueron incubadas con P fueron siempre menores que aquellas incubadas con I o S. Inulina y S tuvieron un efecto similar excepto en la FDN extraída de Alfalfa. Los polisacáridos solubles neutro-detergente no afectarón la cinética de digestión de FND proveniente de forrajes cuando el pH de los cultivos fue mantenido por encima de 6.6 pero si la producción de proteína microbiana *in vitro*. La producción de proteína microbiana *in vitro* promovida por los tratamientos, I y S, fué mayor que la promovida por P.

Palabras clave: Cinética de digestión, fibra, polisacáridos, proteína microbiana.

INTRODUCTION

The effects of neutral detergent-soluble carbohydrates (NDSC) on the kinetics of NDF fermentation by ruminal microbes have provided mixed results. Some reported associative effects were positive [11, 16, 29, 30]. However, most work has shown depressions in fiber digestion which occur as a result of either increased lag time or reduced rate and/or potential extent of digestion [8, 11, 12, 13, 14, 20]. A main factor that leads to decreased ruminal NDF digestion seems to be a ruminal pH under 6.2 [13, 27]. A specific negative carbohydrate effect also known as pH independent negative effect of molasses sugars, starch, or glucose seems in part to depress *in vitro* fiber digestion although findings are controversial [22, 24, 25].

Most of the literature has focused on the effects of starch or glucose as NDSC sources on the fermentation of NDF from alfalfa, temperate grasses, and by-product feeds. Other NDSC are pectins and fructans (fructosans). Fermentation of pectin produces more acetate [6, 31] and, not lactate [31]. Contrary to pectins, fructans can be fermented to lactic acid in the rumen [32]. If ruminal NDF digestibility is affected by NDSC, the energy available for microbial protein synthesis is affected as well. Increased dietary starch or sugars may or may not affect the efficiency of microbial protein synthesis. Currently, few data are available on the effects of pectic substances on microbial growth, and the few studies that exist are contradictory [2, 5, [18]. Neither the effect of pectic substances on digestion of forage NDF, nor the effect of fructosans on either NDF digestibility or microbial protein synthesis has been investigated throughly. Therefore, research to evaluate the effects of NDSC, other than starch, on NDF digestion through kinetic studies is warranted.

The purposes of this experiment are to compare the pH-independent effects of starch, fructans, and pectins, described in this study as neutral detergent-soluble polysaccharides (NDSP), on forage NDF digestion kinetics and microbial protein synthesis. Isolated NDF from alfalfa, bermudagrass, and timothy hay were used as the fiber sources, and inulin, citrus pectin, and corn starch as sources of NDSP.

MATERIALS AND METHODS

Macro-Fiber Preparation

Isolated NDF was prepared from alfalfa, bermudagrass and timothy hays. Forages were ground through the 1-mm screen of a Wiley mill prior to extraction. Forage samples were refluxed with ND solution in a ratio of 1.5 g hay: 50 mL neutral detergent + 0.2 mL heat-stable -amylase for 1 h in a macro NDF reflux system by the method of Smith and Waldo [28]. The NDF was filtered under vacuum though a 37-m pore size nylon cloth set in a large Buchner funnel, and was rinsed repeatedly with boiling hot distilled water. To remove residual ND solution, the extracted sample was then transferred into an appropriate container for overnight incubation in 1 *M* ammonium sulfate solution (approximately 200 mL to 5 g of NDF). The NDF was then filtered under vacuum through the nylon cloth set in a Buchner funnel, and rinsed with boiling hot distilled water until no more foaming from detergent was observed. Finally, the residue was rinsed, washed with acetone, and filtered under vacuum until dry. The NDF then was dried overnight at 55°C in a forced air oven.

Sample Preparation

The NDSP used were corn starch (S; 4126; Sigma Chemical., St. Louis, MO), dahlia tuber inulin (I; 3754; Sigma Chemical., St. Louis, MO), and citrus pectin (P; 9135; Sigma Chemical., St. Louis, MO). These soluble carbohydrates were mixed with the isolated NDF in proportions to produce blends containing approximately 40% NSC. After blended samples were prepared, their actual NDF contents were determined (TABLE I). Isolated NDF without added soluble carbohydrate served as the control. Four hundred mg aliquots of each blended sample were weighed into duplicate 100 mL polypropylene tubes (Nalgene Brand Products, Nalge Co.; Rochester, NY) for the batch fermentations.

T*ABLE I*
ANALYZED COMPOSITION OF SUBSTRATES AND NDSP SOURCES[A] (MEAN±SE)[B]

Substrate	DM %	OM	CP	NDF	Ash
		% of DM			
A	94.64± 0.00	99.35 ±0.04	5.98± 0.15	92.86 ±0.06	0.65 ±0.04
A + I	90.66± 0.04	99.43 ±0.01	….	55.98 ±2.34	0.57 ±0.01
A + P	88.75± 0.03	97.60 ±0.03	….	60.86 ±0.26	2.40 ±0.03
A + S	90.05± 0.04	99.56 ±0.06	….	58.79 ±1.23	0.44 ±0.06
B	93.39± 0.09	99.08 ±0.21	5.68± 0.08	96.31 ±0.27	0.92 ±0.21
B + I	90.43± 0.13	99.24 ±0.04	….	58.49 ±0.81	0.76 ±0.04
B + P	88.98± 0.24	97.96 ±0.31	….	58.88 ±1.77	2.04 ±0.31
B + S	89.83± 0.00	99.06 ±0.05	….	59.68 ±0.50	0.94 ±0.05
T	96.68± 0.09	99.66 ±0.03	3.97± 0.12	93.06 ±0.18	0.34 ±0.03
T + I	90.74± 0.03	99.42 ±0.01	….	57.41 ±0.70	0.58 ±0.01
T + P	89.19± 0.09	98.06 ±0.02	….	61.35 ±1.62	1.94 ±0.02
T + S	90.36± 0.00	99.61 ±0.07	….	58.37 ±0.59	0.39 ±0.07
NDSP					
I	97.68± 0.07	99.74 ±0.01	0.32± 0.01	….	0.26 ±0.01
P	93.62± 0.02	96.44 ±0.04	1.58± 0.08	….	3.56 ±0.04
S	87.34± 0.06	100±0.00	0.30± 0.01	….	0

[a] Isolated NDF sources are: A = alfalfa, B = bermudagrass, T = timothy, and NDSP sources are I = inulin, P = pectin, and S = starch.
[b] Two samples were analyzed for all assays.

Inoculum Donor Cow

Strained rumen fluid from a ruminally fistulated, nonlactating Holstein cow fed 9 kg of DM of a forage and concentrate diet (80:20) was used as inoculum (10 mL/tube). The diet was balanced to meet NRC recommendations [23]. It contained 11.1% alfalfa hay, 44.2% bermudagrass hay, 22.1% timothy hay, 9.9% hominy feed, 9.9% dry citrus pulp, 2.2% soybean meal, and 0.6% mineral mix as a percentage of ration DM. The cow was fed this diet once a day for at least 10 d prior to ruminal sampling.

Evaluation of *in vitro* ndf digestion and microbial protein production

Two separate 96 h *in vitro* batch fermentations (runs) were done for each forage type [10]. Each incubation was conducted in duplicate (separate runs) for a total of 120 tubes [(four treatments + blank) x two replicates x 12 time points] for NDF determinations and three additional tubes per treatment including blanks were incubated for 0 and 24 h for a total of 30 tubes per run [(four treatments + blank) x three replicates x 2 time points] for microbial protein estimation. Tubes were swirled gently immediately after inoculation and at 12 h intervals.

At the end of each incubation time pH was determined on each tube using a pH meter (Accumet model 15, Denver Instrument Co, CO; and Accumet Research model AR15, Fisher Scientific, Pittsburgh, PA), and stored at 4°C to stop fermentation and to be analyzed for NDF. Tubes for microbial protein estimation started to be analyzed for TCA-precipitable nitrogen 20 min after removal from the incubator.

General analysis

Subsamples of isolated NDF were analyzed for DM and NDF-OM (NDF-Ash Free) content. Duplicate sample and blank tubes were analyzed for ash-free NDF at 0, 2, 4, 6, 12, 18, 24, 30, 36, 48, 72, and 96 h incubation according to the method of Van Soest et al. [33], including heat-stable -amylase (Termamyl 20L; Novo Laboratories, Inc., Danbury, CT). The NDF residue at zero hour was determined by analyzing substrates that were inoculated and then immediately removed from the incubator.

TCA-precipitable nitrogen containing both microbial and undigested substrate nitrogen was measured by a modification of the method of Cline *et al*. [7]. Ten milliliters of 60% trichloroacetic acid (w/v)(TCA), (A322, Fisher Scientific., Pittsburgh, PA) were added to each fermentation tube, to provide a final TCA concentration of 10%. After swirling and incubating for 30 min at room temperature, the tube contents were divided into two 50 mL centrifuge tubes. Tubes were centrifuged at 5,000 rpm for 20 min, and the supernatant and the precipitate filtered through filter paper (Whatman 541, 150 mm diameter, Fisher Scientific, Pittsburgh, PA). The 50 mL centrifuge tubes were rinsed onto the filter paper with diluted 3% TCA to remove resi-

dues. The filtrate from the initial filtration was filtered again through glass microfibre filter paper (Whatman GF/A, 125 mm diameter, Fisher Scientific, Pittsburgh, PA). The second filtrate was discarded. All filter papers from a given sample were transferred to a Kjeldahl flask for nitrogen analysis by the macro Kjeldahl method [3]. An equal number of filter papers were included as a blank in the Kjeldahl analysis to correct for their nitrogen content.

Microbial protein estimation

The quantity of microbial protein synthesized in 24 h (TCAP) was estimated as follow:

For each run, TCA-precipitable nitrogen in tubes at 0 h was determined by subtracting the arithmetic mean of the blanks (media + inoculum) from TCA-precipitable nitrogen of treatment tubes at 0 h. Least squares means for the blank-corrected treatment values at 0 h were calculated. The final TCA-precipitable nitrogen (microbial nitrogen synthesized and residual substrate nitrogen) in tubes at 24 h was estimated by subtracting the arithmetic mean of the blank for 24 h from TCA-precipitable nitrogen of each treatment tube at 24 h. The change in the TCA-precipitable nitrogen expressed as crude protein (TCAP) was calculated as the difference between 24 h and 0 h TCA-precipitable nitrogen multiplied by 6.25. It is interpreted as an estimate of the amount of microbial protein synthesized during 24 h of incubation.

Kinetics analysis

Neutral detergent fiber disappearance data were fitted to the model of Mertens [19, 20].

$Y = D_0e^{-Kd(t-L)} + INDF$, when $t>L$ and $Y = D_0 + INDF$ when $0<t<L$

Where:

Y = NDF residue at time t (time after inoculation),

D_0 = potentially digestible NDF fraction (at time t L, D_0 = Y- INDF),

Kd = fractional rate constant of digestion (per hour),

L = discrete lag time (h),

t = time (h), and

INDF = indigestible NDF fraction

$L = (\ln D_0 - \ln D'')/-Kd$,

Where:

D" = intercept of the equation of ln (Y - 96h residue) over time.

For individual forage NDF sources, all four dependent variables (D_0, INDF, L, and Kd) were calculated iteratively for each treatment within a run by Marquardt's compromise by procedure NLIN of SAS [26].

Potential extent of NDF digestion (PED), the percentage of total NDF that is potentially digestible, was calculated by using the equation from Grant and Mertens [13].

$PED = 100 \times D_0/(D_0 + INDF)$.

The predicted apparent extent of NDF digestion in the rumen (PAERD), the percentage of PED digested in the rumen, was calculated for each run (kinetic parameters for each run) by the equation from Miller and Muntifering [21].

$PAERD = PED \times e^{-KpL} \times Kd/(Kd + Kp)$.

Where PAERD = predicted apparent extent of ruminal NDF digestibility and

(Kp) = rates of passages which were assumed as 0.02, 0.04, and 0.06 h^{-1} to represent low, medium, and high feed intakes respectively.

Statistical analysis

The experiment was performed as a randomized complete block design for each individual forage source. For each isolated NDF source, kinetic data (lag time, rate, potential extent of digestion) or predicted apparent extent of ruminal NDF digestibility were analyzed with each *in vitro* digestion run (n = 2) as experimental units arranged by treatments (n = 4). Interaction effect (run x treatment) could not be evaluated because only one value for each parameter within a run was achieved.

The TCAP data was arranged as a 4 x 2 factorial of treatments and runs (including the interaction "run x treatment") in a randomized complete block design.

The pH data from each forage's NDF fermentations were analyzed by utilizing the test for heterogeneity of regression. After attaining a significant F-statistic (P =0.01) for the test, regression parameters were estimated for each treatment of individual forage NDF, including both runs, to predict values from 0 to 96 hours and to draw regression curves. Only pH data from 0 to 48 h incubation were included in the analyses of individual pH treatment curves of separated runs. Effect of hour on pH curves for each treatment within NDF forage source was tested for higher order polynomials by including first, second, third, fourth, and fifth order interaction terms in the model. Regression parameter estimates for each treatment were obtained by a final model that included the effects of treatment, treatment x hour, treatment x $hour^2$, and treatment x $hour^3$. Predicted pH values were calculated every 30 min from 0 to 48 h by regression equations of each treatment and run. The predicted pH at 0 h, the lowest predicted pH, the change in pH between 0 h and lowest pH (pH), and hour at which the lowest pH was reached were analyzed with each *in vitro* digestion run (n = 2) as experimental units arranged by treatments (n = 4). Interaction effect (run x treatment) could not be evaluated because only one value for each dependent variable within a run was achieved.

For each isolated NDF source, the effects of NDSP on either kinetic variables, predicted apparent extent of ruminal

NDF digestibility, TCAP, pH at 0 h, the lowest pH, pH, and hour at the lowest pH were compared by using Student-Newman-Keuls multiple range test. Significance was declared at $P<0.05$ unless otherwise stated.

All analyses were performed using the general linear models procedures of SAS [26].

RESULTS AND DISCUSSION

pH of fermentations cultures

The pH curves for the different NDSP treatments were not parallel ($P = 0.01$) (FIGS. 1, 2, 3) which mean that they differed among NDSP for all NDF sources. Across all NDF forage sources, pH values decreased within 24 h, tended to increase thereafter, and reached values greater than initial after 48 h fermentation.

Although each forage NDF source was analyzed as a separate experiment, results of the effects of NDSP on fermentation pH are depicted together for convenience (TABLE II). For all forage NDF sources, P had a lower initial pH as compared to the other treatments. Overall, the NDSP dropped the pH of incubation fluids as compared to the control without differences across NDSP. Both the ΔpH value and hour at which the lowest pH was reached were not affected by treatments. The addition of either I or S to alfalfa NDF tended to produce a greater change in pH value as compared to the control whereas the addition of pectin to timothy NDF tended to produce a lower change in pH value than the other treatments ($P<0.1$). For bermudagrass as well as for timothy, P tended to reach the lowest pH sooner than I, S, and C ($P<0.1$). Therefore, these results suggest that pectin fermented more rapidly than starch. However, we did not measured pectin degradation in this study. The ΔpH could also indicate the extent of NDSP fermentation since pH of incubation fluid is reduced by both organic acids and CO_2 produced from fermentation. The lower ΔpH for P might indicate a lower extent of fermentation. Organisms that degrade pectin may be especially pH sensitive [31]. Therefore, it is possible that a rapid drop on pH of ferments containing pectin had repressed further fermentation.

Effect of NDSP on microbial protein production

The effects of NDSP addition to forage NDF on estimated microbial crude protein synthesized *in vitro* (ΔTCAP) are in TABLE III. For all forage sources, both TCAP (mg/24 h) and efficiency of microbial crude protein production expressed as milligram of TCAP produced per gram of substrate OM (ΔTCAP mg/OM g) were affected by NDSP. For alfalfa NDF, I promoted the highest microbial protein production followed by S and P. The efficiency of ΔTCAP production was higher for I than for either S or P. The efficiency for P was similar to that of S. For bermudagrass and timothy, ΔTCAP production and efficiencies for I plus S were greater than that of C or P. Starch and I had similar efficiencies of microbial protein production *in*

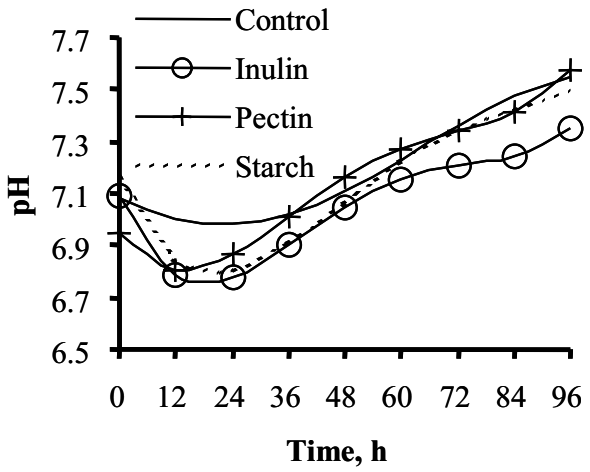

FIGURE 1. REGRESSION CURVES FOR PH OF INCUBATION FLUID FROM FERMENTATIONS OF ALFALFA NDF (MEAN OF TWO RUNS).

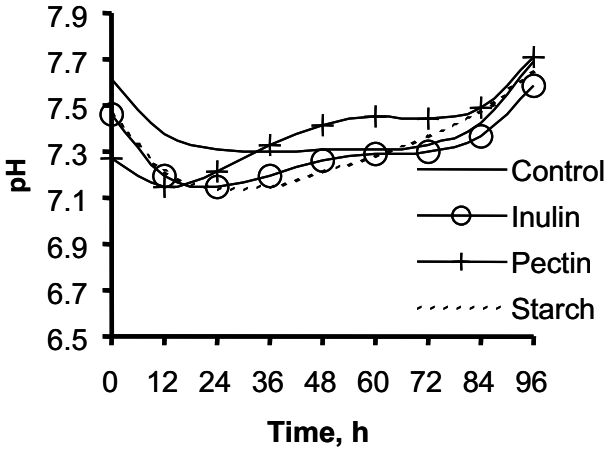

FIGURE 2. REGRESSION CURVES FOR PH OF INCUBATION FLUID FROM FERMENTATIONS OF BERMUDAGRASS NDF (MEANS OF TWO RUNS).

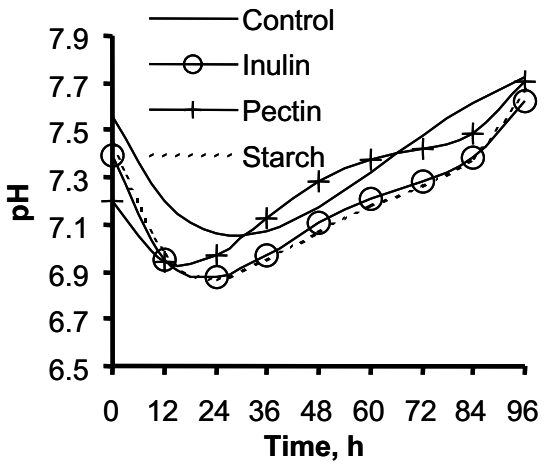

FIGURE 3. REGRESSION CURVES FOR PH OF INCUBATION FLUID FROM FERMENTATIONS OF TIMOTHY NDF (MEAN OF TWO RUNS).

TABLE II

EFFECTS OF NDSP[1] ON PH OF INCUBATION FLUIDS FROM ISOLATED FORAGE NDF FERMENTATION (LSMEANS ± SE) [2]

	Forage NDF Source														
	Alfalfa					Bermudagrass					Timothy				
	NDSP added[3]														
pH	C	I	P	S	SE	C	I	P	S	SE	C	I	P	S	SE
Initial	7.09[a]	7.09[a]	6.98[b]	7.16[a]	0.015	7.60[a]	7.47[ab]	7.29[b]	7.47[ab]	0.040	7.56[a]	7.42[a]	7.23[b]	7.46[a]	0.035
Lowest	6.97[a]	6.75[b]	6.78[b]	6.80[b]	0.030	7.31	7.15	7.15	7.14	0.029	7.06[a]	6.87[b]	6.91[b]	6.87[b]	0.011
ΔpH[4]	0.12	0.34	0.20	0.36	0.044	0.29[a]	0.32[a]	0.14[b]	0.33[a]	0.019	0.50	0.55	0.32	0.59	0.046
h at lowest pH	18.75	16.75	10.25	18.75	1.85	22.00	16.50	8.25	22.50	2.12	23.75[a]	17.50[b]	12.25[c]	18.50[b]	0.35

[abc]LSMeans within a forage NDF source in each row differ (P<0.05).
[1]Neutral detergent-soluble polysaccharides.
[2]Values are least squares means of two estimates (one on each of two runs) by regression equation pH = trt trt*h trt*h2 trt*h3.
[3]C= isolated NDF, I = C plus inulin, P = C plus pectin, S = C plus starch.
[4]ΔpH= Change in pH from 0 h to the hour of lowest pH.

TABLE III

EFFECTS OF NDSP[1] ON ΔTCAP[2] OF ISOLATED FORAGE NDF FERMENTATIONS (LSMEANS ± SE) [3]

	Forage NDF Source											
	Alfalfa				Bermudagrass				Timothy			
	ΔTCAP[5], mg		ΔTCAP[6], mg/g OM		ΔTCAP, mg		ΔTCAP mg/g OM		ΔTCAP, mg		ΔTCAP mg/g OM	
NDSP added[4]	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C	13.07[d]	1.64	32.86[c]	4.29	11.58[b]	1.21	29.26[b]	3.14	16.14[b]	1.73	40.48[b]	4.51
I	28.55[a]	1.46	75.95[a]	3.84	20.30[a]	1.21	53.75[a]	3.14	29.61[a]	1.73	79.77[a]	4.51
P	18.58[c]	1.46	50.62[b]	3.84	9.20[b]	1.21	24.70[b]	3.14	18.75[b]	1.73	51.30[b]	4.51
S	23.17[b]	1.46	59.29[b]	3.84	20.06[a]	1.21	51.24[a]	3.14	26.71[a]	1.73	68.81[a]	4.51

[abcd]LSMeans within a column with different superscripts differ (P< 0.05). [1]Neutral detergent-soluble polysaccharides.
[2]Change in trichloroacetic acid-precipitable protein (TCAP).
[3]Values are least squares means.
[4]C= isolated NDF, I = C plus inulin, P = C plus pectin, S = C plus starch.
[5]ΔTCAP, mg = Change in TCAP (N*6.25) from 0 to 24 h.
[6] ΔTCAP, mg/g OM = Milligrams of ΔTCAP/g of substrate organic matter.

vitro when fermented with either bermudagrass NDF or timothy NDF. Early studies reported ΔTCAP (mg/30 h) values for cellulose of 22 mg/g of substrate [7, 15]. For blends of cellulose and starch, the values rose to 62 to 66 mg/g of substrate [7]. In general, the values of ΔTCAP (mg/24 h) for C and S in all forage sources of the current study appeared close to those previously reported. Barrios-Urdaneta et al. [5] found starch most effective in promoting bacterial growth at 8 h of *in vitro* incubation with isolated NDF from barley, followed by pectin, sugars, and the control cultures. In general, our results for P and S are in agreement with those of Barrios-Urdaneta et al. [5]. The ability of P to stimulate microbial protein synthesis when added to alfalfa NDF but the failure when added to either bermudagrass or timothy NDF is difficult to explain. The effect of I on microbial protein synthesis has not been reported previously. The lower ΔTCAP values for P as compared to I or S seem contrary to the predictions that microbial yield should be more similar, or higher for P based on rates of fermentation [33]. This result is difficult to interpret because other indicators of the extent of fermentation such as VFA and lactate production are lacking. It can be assumed that organic acids and CO_2 produced from fermentation decrease pH while increases in pH would be due to reduction in acid load (VFA or CO_2 escape) or addition of base, possibly from ammonia resulting from microbial recycling. Therefore, the pH nadir could indicate the point at which the NDSP fermentation has been maximized. Then, substrate depletion would occur and microbial death/recycling be increased. In the present study, the pH of P tended to reach its nadir before I and S, except for alfalfa. However, there was not difference in the pH nadir per se. Furthermore, P added to forage NDF resulted in a lower change in pH value than the other NDSP with the exception of alfalfa NDF. If fermentation of added pectin was greater than that of the other imposed treatments, then P would affect the change in pH to a greater ex-

tent than the other NDSP. However, this hypothesis is refuted because P produced a lower change in pH value than the other NDSP in the case of both bermudagrass and timothy NDF fermentations. *In vitro* studies on continuous culture suggest that the period of time that pH is below optimal (6.3) may be more critical for grass digestion than the sub-optimal mean pH per se [34]. Although in the present study, pH stayed above level considered as optimal (6.6), a reduction in TCAP could have occurred because P treatment stayed with lowest pH much longer as compared to the other treatments. These results suggest that the comparison of TCAP among NDSP at a fixed time point in batch culture may not accurately reflect their potential microbial protein production.

Digestion Kinetics of Forage NDF

Alfalfa NDF. The effects of NDSP on forage NDF digestion kinetics are assumed to be pH independent [22, 24] because fluid pH did not decrease below 6.6 at any time of fermentation. The NLIN Marquardt's procedure of SAS set to estimate iteratively all four kinetic parameters at once was found to accurately fit the observed fermentation data of all of the NDF forage sources to the first order model of Mertens [19]. By observing fitted curves (not shown), digestion of NDF from the alfalfa blends appeared to be essentially complete by 48 h. Lag time was not affected by the presence of NDSP in the mixtures (TABLE IV). Overall, fractional rate of digestion was not affected by the presence of NDSP, but Kd for P tended to be lower than S ($P < 0.1$) (not shown in TABLE). The addition of NDSP did not affect the potential extent of digestion in any blend. In the current study, estimates of lag are longer than those reported by other workers using an *in vitro* system adjusted to a pH of 6.8 with [11, 13] or without [14] starch additions and no change in lag was observed on alfalfa hay with any starch addition [11]. Lag time may [1] or may not be affected by microbial adhesion [9]. Recent studies with mixed rumen bacteria have shown that added pectin increased both the proportion of adherent bacteria [5] and the rate of adhesion [4] to isolated NDF from barley when compared to starch or a mixture of soluble sugars (glucose, maltose, fructose, and sucrose). Reports indicate that rate (Kd) of forage NDF digestion decreased [11] or not [13, 14] when starch was added to mixed cultures that had a pH >6.2. Piwonka and Firkins [24] found that addition of glucose decreased the Kd of corn bran NDF as compared to the control when pH of the medium remained greater than 6.2. In the present study, Kd of the control did not differ from that of NDSP, but Kd for P tended to be lower than that of S ($P < 0.1$). This result suggests that pectin and starch may affect the Kd differently for alfalfa NDF when added at 40%. Research is needed to confirm this trend.

Bermudagrass NDF. Digestion of NDF in the bermudagrass blends appeared to be essentially complete by 72 h. The presence of NDSP did not alter the lag time, Kd, or the PED of bermudagrass NDF (TABLE IV). The lag time for isolated NDF from Tifton 85 bermudagrass harvested after 3.5 wks of growth, a second cutting after 3.5 wks of regrowth, 7 wks of growth, and from costal bermudagrass harvested after 4 wks of growth were 1.88, 2.0, 2.75, and 1.45 h, respectively [17]. Our estimates of L were much greater than those. Our results are similar to those presented by Mertens and Loften [20] who found that starch additions ranging from 0 to 80% as fed substrate did not affect lag time, Kd, or PED of coastal bermudagrass at pH of 6.8.

Timothy NDF. For isolated timothy NDF, the effects of NDSP on *in vitro* digestion kinetics of NDF are in TABLE IV. Digestion of timothy NDF appeared to be complete by 48 h. The presence of NDSP did not affect L, Kd or PED as compared to the control timothy NDF. There were not differences among treatments. Some inhibition of forage NDF digestibility is expected due to a specific negative carbohydrate effect, even at pH 6.8. Grant and Mertens [13] found that addition of raw corn starch decreased NDF digestibility by 23% for alfalfa hay and bromegrass hay at pH 6.8. Piwonka and Firkins [24] found that glucose inhibited fiber digestion separate from the effects of pH as shown by an *in vitro* study using cellulose and soyhulls as cellulosic fiber sources, and corn bran as a hemicellulosic fiber source. However, the same researchers did not find any reduction in the NDF digestion rate of combined data of cellulose, soybean hull and corn bran fermented in a fresh glucose medium (25 m*M* glucose) at pH over 6.2 [25]. Grant [11] indicated that forage type must be considered when the effect of pH and starch source on NDF digestion is predicted. Therefore, the results from the present study suggest that there are not detrimental effects of polysaccharides on kinetics of NDF digestion when pH is maintained over 6.6.

Predicted Apparent Extent of Ruminal NDF Digestibility

Alfalfa NDF. For alfalfa substrates, the predicted apparent extent of NDF digestion in the rumen (PAERD) based on *in vitro* digestion kinetics obtained from each run are in TABLE IV. In the present study, the PAERD were not affected by the NDSP. Predicted apparent extent of ruminal NDF digestibility of C, I and S were between 70 and 74 percent of the PED for a low rate of passage. Pectin had a PAERD of about 64% of the PED at the low passage rate because the term Kd/(Kd+Kp), which represents the theoretical maximum proportion of NDF disappearance from the rumen has a relatively low value as compared to the term e^{-KpL}. In general, those calculated values for PAERD are comparable to other measures of NDF digestion observed by *in vitro* studies. At pH of 6.8, Grant and Mertens [13] calculated PAERD using 0.02 h^{-1} Kp to be 26.1% and 33.1% for alfalfa NDF with and without addition of raw corn starch, respectively. At similar pH and assuming a 0.05 h^{-1} Kp, the calculated PAERD were 28.3% and 31.1% for alfalfa and bromegrass NDF, respectively [14]. For alfalfa hay mixed with different starch sources, the calculated PAERD using a 0.02

TABLE IV

EFFECTS OF NDSP[1] ON DIGESTION KINETICS AND PREDICTED APPARENT EXTENT OF RUMINAL NDF DIGESTIBILITY (PAERD) OF ISOLATED FORAGE NDF (LSMEANS ± SE)

	Forage NDF Source														
	Alfalfa					Bermudagrass NDSP added[2]					Timothy				
Item	C	I	P	S	SE	C	I	P	S	SE	C	I	P	S	S
Digestion Kinetics[3]															
Lag (h)	6.73	7.72	0.07	8.03	2.85	7.02	7.64	8.86	10.27	2.14	6.80	6.41	3.71	7.23	1.46
Kd (h^{-1})[4]	0.084	0.099	0.049	0.139	0.013	0.040	0.035	0.026	0.062	0.013	0.069	0.077	0.069	0.088	0.007
PED%[5]	42.45	42.07	52.12	39.84	6.83	64.95	56.66	65.12	62.05	4.60	56.31	49.12	59.00	55.86	3.71
PAERD[8]															
0.02 h^{-1}	29.92	30.40	33.32	29.64	3.50	36.81	30.26	27.70	37.80	1.63	38.02	33.89	42.35	39.21	1.50
0.04 h^{-1}	21.97	23.11	25.01	22.54	3.18	24.09^{a}	19.13^{b}	16.06^{b}	24.76^{a}	0.74	27.07^{b}	24.62^{b}	32.04^{a}	28.64^{b}	0.72
0.06 h^{-1}	16.61	18.29	20.15	17.45	3.12	16.93^{a}	13.15^{b}	10.33^{c}	16.99^{a}	0.50	19.97^{ab}	18.50^{b}	25.10^{a}	21.50^{ab}	0.89

^{abc}LSMeans within a forage NDF source in each row differ ($P<0.05$).
[1]Neutral detergent-soluble polysaccharides.
[2]C= isolated NDF, I = C plus inulin, P = C plus pectin, S = C plus starch.
[3]Values are least squares means of two parameter estimates (one on each of two runs). All parameters (PED, Lag, k_d, and INDF) were iteratively calculated by NLIN Marquardt's compromise procedure of SAS.
[4] Kd = Fractional rate of NDF digestion (per hour).
[5]PED = Potential extent of digestion, calculated as $100*D_0/(D_0 + INDF)$, where D_0 = potentially digestible NDF (percentage of sample NDF), and INDF = indigestible NDF (percentage of sample NDF) according to Grant and Mertens (1992b).
[6]LSMeans of two runs. PAERD = PED x e-KpL x Kd/(Kd + Kp) according to Miller and Muntifering, 1985.

h^{-1} Kp were 35.4, 32.9, and 30.2% for raw sorghum, raw corn, and pure corn starch additions, respectively [11].

Bermudagrass NDF. The predicted apparent extent of NDF digestion in the rumen (PAERD)(TABLE IV) were between 60 and 50 percent of the PED (TABLE IV) for a 0.02 h^{-1} Kp across treatments except for P which reached only 43 percent of the PED. The PAERD for P would indicate that 57% (100% of the potentially extent of digestion in the rumen - 43%) of the PED escaped undigested from the rumen because of passage before digestion began. The predicted apparent extent of NDF digestion in the rumen for bermudagrass mixed with S was greater than that of bermudagrass mixed with I or P at all rates of passage except at 0.02 h^{-1}. Starch had no effect on PAERD as compared to C at any of the passage rates tested. Both I and P decreased PAERD as compared to C at both 0.04 to 0.06 h^{-1} Kp. This negative effect was greater for P than for I when rate of passage was 0.06 h^{-1}. These results suggest that S would not stimulate PAERD, but either I or P could reduce it. This negative effect could be greater for P than for I as Kp increases.

Timothy NDF. The predicted apparent extent of NDF digestion in the rumen based on *in vitro* digestion kinetics are in TABLE IV. Predicted apparent extent of ruminal NDF digestibility were between 68 and 72 percent of the PED for the 0.02 h^{-1} Kp across all treatments. In the present study, the PAERD were not affected by S and I at any of the passage rates tested. Pectin stimulated PAERD at medium Kp (0.04 h^{-1}) passage rate. Pectin had greater PAERD than I at both 0.04 h^{-1} and 0.06 h^{-1} passage rates, but only had greater PAERD than S at 0.04 h^{-1}. The predicted apparent extent of NDF digestion in the rumen for I was similar to that of S at all rates of passage. There is not a clear explanation of why P decreased PAERD for bermudagrass and caused a positive effect on the same variable for timothy. Recent studies with mixed rumen bacteria have shown that added pectin increased both the proportion of adherent bacteria [5] and the rate of adhesion [4] to cell walls when compared to starch or a mixture of soluble sugars (glucose, maltose, fructose, and sucrose). Therefore, a positive associative effect of pectin on PAERD by shortening lag might be caused by adhesion mechanisms of the plant cell-associated ruminal microbes. More research is needed to compare the effects of pectin on digestion kinetics of different forage sources.

CONCLUSIONS

The neutral detergent-soluble polysaccharides effects on forage NDF fermentation when pH of cultures were maintained over 6.6 were as followed: Neither Lag times nor potential extent of digestion of alfalfa, bermudagrass, and timothy NDF were affected by NDSP. For alfalfa NDF, Kd for P tended to be lower than that of S ($P<0.1$). The calculated values of predicted apparent extent of ruminal NDF digestibility were not altered by the addition of NDSP to alfalfa NDF. Both I and P additions to bermudagrass NDF had negative effects on the PAERD at medium and high Kp. However, pectin addition to timothy NDF had a positive effect on the PAERD at medium Kp. At the end

of the 24 h fermentation, the positive effect of NDSP on estimated microbial CP yield was more pronounced for I added to forage NDF. Starch and I had similar TCAP except when fermented with alfalfa NDF. The estimated microbial CP yield for P was always less than I and S. For alfalfa NDF, however, pectin addition stimulated TCAP. Although the results by forage type are not directly comparable, there appear to be differences in the effects of NDSP by NDF source.

ACKNOWLEDGMENTS

The financial support of Universidad Centroccidental "Lisandro Alvarado" and CONICIT (Venezuela) is greatly appreciated. We are also very grateful to Luis Carlos Freitez for his technical assistance.

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