Effects of the source of non-fiber carbohydrates on *in vitro* first order ruminal disappearance kinetics of dry matter and NDF of various feeds

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Summary

An *in vitro* experiment was conducted to study the effect of non-fiber carbohydrates (NFC) on ruminal disappearance kinetics of dry matter (DM) and neutral detergent fiber (NDF) of alfalfa hay, wheat bran and unmolassed sugar beet pulp. Non-supplemented or NFC supplemented samples [70 mg/g DM of each feed sample as sucrose (SU) or starch (ST) or SU + ST as 1:1] were incubated in bottles containing 40% rumen fluid medium for 4, 8, 16, 24, 48, and 96 h at 39°C. After each incubation time, unfiltered content was analysed for DM and NDF. Filtered fluid was analysed for ammonia-N concentration. Kinetic disappearance rate was determined using a first order non-linear model. The source of NFC used in the medium containing alfalfa hay caused a significant decrease in DM disappearance (P<0.01). Feed source, NFC type and incubation time had a significant effect (P<0.01) on NDF disappearance and ammonia-N concentration. *In vitro* NDF disappearance of unmolassed sugar beet pulp and wheat bran was significantly (P<0.01) decreased by supplementing with ST and SU + ST. The indigestible dry matter fraction of alfalfa hay was significantly increased when it was supplemented by NFC (P<0.05). The rate of DM disappearance of unmolassed sugar beet pulp was also significantly influenced by supplemental NFC (P<0.05). The fractional rate of NDF disappearance of alfalfa hay and wheat bran exhibited an increase (P<0.05) in response to adding NFC when compared with the non-supplemented samples. Generally, the results of the present study suggested that in addition to the amount of supplemental carbohydrate fed, the source of supplemental carbohydrate and the basal feed sources might be effective on the effect of supplemental NFC on fiber utilization.

Key words: Disappearance, Starch, Sucrose, Rumen fluid

Introduction

A current feed evaluation study discovered that ruminal disappearance kinetics of a nutrient varied among the feed sources evaluated (Jahani-Azizabadi *et al.*, 2009; Abdi Ghezeljeh *et al.*, 2011). Previous results suggested that partial replacement of barley grain with dried molassed sugar beet pulp at low and moderate inclusion rates might improve the chewing behavior, ruminal environment and nutrient digestibility of Holstein steers fed low-forage diets (Mojtahedi and Danesh Mesgaran, 2011). It was also suggested that supplementing forage-based diets with feedstuffs that contain high concentrations of rapidly digestible non-fiber carbohydrates (NFC) including starch (ST) and sucrose (SU) decreased forage fiber disappearance (Heldt *et al.*, 1999a). Fondevila *et al.* (2002) suggested that the addition of carbohydrates such as starch negatively affected the *in vitro* microbial fermentation of straw, even at optimum pH. The relative impact on fiber disappearance of supplementation with sugars versus starch is of particular interest (Heldt *et al.*, 1999a).

Substitution of ST with water soluble sugar might affect ruminal ammonia-N concentration. Increasing the supply of ruminally fermentable carbohydrates reduced ruminal ammonia-N concentration (Rezaii *et al.*, 2008). In addition, previous results showed a reduction in ruminal ammonia-N concentration when sugar was included in the ration (Lee *et al*., 2003). Khezri *et al*. (2009) found that increasing levels of sucrose in diets compared with corn starch reduced mean ruminal ammonia-N concentration. Chamberlain *et al*. (1985) reported that SU was more effective than ST in reducing ruminal ammonia-N concentration. Very little quantitative information is available on the effect of SU or its monosaccharide constituents on the digestion of forages or non-forage NDF sources (Heldt *et al*., 1999a). The effective use of high-sugar products in supplementation programs requires knowledge of their effects on forage used and of how they compare with other common supplemental carbohydrate sources such as ST (Heldt *et al*., 1999a). The objective of this study was to determine the effect of supplementing SU and/or ST on *in vitro* dry matter (DM) and NDF disappearance kinetics of alfalfa hay, unmolassed sugar beet pulp and wheat bran.

Materials and Methods

The present experiment was conducted in the animal nutrition laboratory in Ferdowsi University of Mashhad in order to elucidate the relative importance of carbohydrate type as well as feed source on both DM and NDF disappearance by mixed ruminal microorganisms. Alfalfa hay was chosen as a representative legume, and both wheat bran and unmolassed sugar beet pulp were selected as typical highly digestible NDF sources. Chemical compositions of feedstuffs are shown in Table 1.

Substrates and medium preparation

Feed samples were ground using a Willey-mill to pass 0.75 mm screen, and oven dried at 60°C for 48 h. The fermentation medium was prepared according to Arroquy *et al*. (2005), and included 400 ml cell-free ruminal fluid, cellobiose (0.05 g), K_2HPO_4 (0.45 g), KH_2PO_4 (0.45 g), NaCl (0.90 g), $(NH_4)_2SO_4$ (0.90 g), $MgSO_4 \cdot 7H_2O$ (0.09 g), $CaCl_2$ (0.09 g), resazurin (0.01 g), $NaHCO_3$ (4 g), and cysteine-HCl (0.5 g) per liter of medium. Rumen fluid was obtained from four Holstein steers fed corn silage, alfalfa hay, wheat straw, barley grain and soybean meal (3.4, 2.4, 0.8, 1.6 and 0.8 kg/d DM, respectively), strained through 4 layers of cheesecloth, and centrifuged at 3000 RPM for 5 min. Then, the supernatant was centrifuged at 15000 RPM for 15 min. Forty-five ml of the medium was transferred into a 100-ml bottle containing the experimental sample and autoclaved at 120°C for 20 min. Each bottle was then inoculated with 5 ml of cheesecloth strained ruminal fluid and finely bubbled with CO_2, sealed and incubated. Previous to the inoculation, the ruminal fluid was incubated for 1 h in an incubation chamber at 39°C (to allow large feed particles to rise to the top), and in time introducing inoculum taking care not to include the large particles that rose to the top nor that which sedimented in the bottom, and was introduced anaerobically into the fermentation bottles.

Experimental procedures

Non-supplemented or NFC supplemented samples were incubated for 4, 8, 16, 24, 48, and 96 h at 39°C (3 bottles per sample and time, n=216). The supplementation was carried out at 70 mg/g DM of each feed sample as SU or ST or SU + ST (as 1:1). After each incubation time, the content of each bottle was filtered through a 22-µm filter paper. The dry matter was determined (60°C, 48 h), then analysed for NDF content (Van Soest *et al*., 1991). Sodium sulfite was used in the NDF analyses, without a heat stable amylase, and expressed including residual ash. The filtered fermentation fluid of incubation of 4, 8, 16 and 48 h was analysed for ammonia-N concentration using steam distillation method (Kjeltec Auto 1030 Analyser, Tecator, Hoganas, Sweden). The feedstuffs were analysed for crude protein (CP) using Kjeldahl procedure (AOAC, 1990), and the ash content was determined after burning at 550°C for 6 h (AOAC, 1990).

Statistical analysis

A non-linear first order model was used to estimate the first order disappearance kinetic parameters of DM and NDF. The model was:

$D_{(t)} = D_{(i)}.\ e^{(-k.\ time)} + I$;

In which,
$D_{(t)}$ = potentially digestible residues
$D_{(i)}$ = potentially digestible fraction
K = fractional rate constant of disappearance (/h)
I = indigestible fraction

Data of ruminal disappearance of the incubations were statistically (SAS, 2003) according to the following model and the comparisons between the main effects were performed using Tukey's test at P<0.05.

$y_{ijk} = \mu + block_i + A_j + (block*A)_{ij} + B_k + (AB)_{ik} + block_i*B_k(A)_j + C_l + (AC)_{jl} + (BC)_{kl} + (ABC)_{jkl} + \varepsilon_{ijkl}$

In which,
y_{ijkl} = the dependent variable
μ = the overall mean
$block_i$ = repeat for each treatment in each time
A_j = main effect of feed source
$(block*A)_{ij}$ = error for estimating A_j
B_k = main effect of NFC
$(AB)_{ik}$ = interaction between NFC type and feed source
$block_i*B_k(A)_j$ = error for estimating B_k and $(AB)_{ik}$
C_l = main effect of incubation time
$(AC)_{jl}$ = interaction between incubation time and feed source
$(BC)_{kl}$ = interaction between NFC type and incubation time
$(ABC)_{jkl}$ = interaction between NFC type and feed source and time incubation
ε_{ijkl} = residual error

First order ruminal *in vitro* disappearance parameters were statistically compared using a completely randomized design (SAS, 2003).

Results

Dry matter and NDF disappearance

Dry matter disappearance is shown in Table 2. The dry matter disappearance of the feed sources was significantly (P<0.01) different. Supplementation of alfalfa hay with NFC reduced DM disappearance significantly (P<0.01), whereas, NFC supplementation had no effect on other feeds. Interactions (P<0.01) were observed between NFC type × incubation time and between NFC type × feed source. A significant three-way interaction (i.e., NFC type × feed source × incubation time) was found for DM disappearance. Generally, NFC supplementation decreased DM disappearance only in alfalfa hay, and in this regard, SU exerted a more negative effect than ST.

The NDF disappearance of alfalfa hay, unmolassed sugar beet pulp and wheat bran supplemented with SU, ST or SU + ST is shown in Table 3. On average, the extent of NDF disappearance was significantly greater (P<0.01) for the non-supplemented feed sources. *In vitro* NDF disappearance of unmolassed sugar beet pulp and wheat bran was significantly decreased by supp-lementing with ST (P<0.01), although it was not observed when alfalfa hay was incubated. A significant effect of adding SU + ST on *in vitro* NDF disappearance of the feed sources was observed, but SU supplementation had no effect on NDF disappearance. Various interactions, namely NFC type × incubation time, feed source × incubation time, NFC type × feed sources and NFC type × feed source × incubation time were significant (P<0.01).

First order DM and NDF fractional rate constant of digestion and indigestible fraction

The effect of NFC types on *in vitro* first order DM and NDF fractional rate constant of digestion and indigestible fraction of the feeds are shown in Table 4. Fractional rate constant of DM disappearance of unmolassed sugar beet pulp and wheat bran was significantly decreased by supplemental NFC (P<0.05), except when SU was added to wheat bran. The fractional rate of NDF disappearance for alfalfa hay and wheat bran (P<0.05) increased in response to NFC supplementation. Sucrose increased the fractional rate of the NDF disappearance of alfalfa hay, but had little effect on unmolassed sugar beet pulp or wheat bran. Starch increased the fractional rate of NDF disappearance for alfalfa hay and wheat bran, but had no significant effect on unmolassed sugar beet pulp. The indigestible fraction of DM of alfalfa hay was significantly increased when it was supplemented with NFC (P<0.05). When SU was added to wheat bran, DM indigestible fraction increased significantly (P<0.05).

Table 1: Chemical composition (g/kg DM) of the feeds evaluated

Nutrient	Alfalfa hay	Unmolassed sugar beet pulp	*Wheat bran*
Crude protein	161	110	*156*
Neutral detergent fiber	540	580	*490*
Ether extract	42	24	*46*
Ash	*53*	*42*	*23*

Table 2: Least square mean of *in vitro* DM disappearance (g/kg) of alfalfa hay, unmolassed sugar beet pulp and wheat bran as non-supplemented or supplemented with starch (70 mg/g DM), sucrose (70 mg/g DM) or starch + sucrose (35 mg of each source per g DM)

Feed	NFC sources						*P-values*[£]		
	Non-supplemented	Sucrose	Starch	Sucrose + Starch	Main mean of the feed samples	SEM	Feed effect	NFC effect	*Time effect*
Alfalfa hay	465[a]	431[b]	437[ab]	411[b]	436[a]	7.1	<0.01	0.169	*<0.01*
Sugar beet pulp	607	613	604	600	606[b]				
Wheat bran	620	625	619	630	623[c]				
Main mean of the NFC source	*564[a]*	*556[ab]*	*553[ab]*	*547[b]*					

[£]P=0.037 for feed source × NFC type, P<0.01 for feed source × incubation time and feed source× NFC type × incubation time, P=0.026 for NFC type × incubation time. [abc]Means within each row and the column of main mean of the feed sample with different letters differ significantly (P<0.01). SEM = Standard error of mean

Table 3: Least square mean of *in vitro* NDF disappearance (g/kg) of alfalfa hay, unmolassed sugar beet pulp and wheat bran as non-supplemented or supplemented with starch (70 mg/g DM), sucrose (70 mg/g DM) or starch + sucrose (35 mg of each source per g DM)

Feed	NFC source						*P-values*[£]		
	Non-supplemented	Sucrose	Starch	Sucrose + Starch	Main mean of feed samples	SEM	Feed effect	NFC effect	*Time effect*
Alfalfa hay	269[a]	252[a]	261[a]	164[b]	236[a]	6.1	<0.01	<0.01	*<0.01*
Sugar beet pulp	411[a]	419[a]	366[b]	370[b]	391[b]				
Wheat bran	525[a]	549[a]	476[b]	491[b]	510[c]				
Main mean of the NFC source	*401[a]*	*406[a]*	*367[b]*	*341[c]*					

[£]P<0.01 for feed source × NFC type, feed source × incubation time, NFC type × incubation time and feed source × NFC type × incubation time. [abc]Means within each row and the column of main mean of the feed samples with different letters differ significantly (P<0.01). SEM = Standard error of mean

Table 4: First order dry matter fractional rate constant of digestion (h^{-1}) and indigestible fraction (mean±SE) of dry matter and NDF of alfalfa hay, unmolassed sugar beet pulp and wheat bran as non-supplemented or supplemented with starch (70 mg/g DM), sucrose (70 mg/g DM) or starch + sucrose (35 mg of each source per g DM)

Feeds	NFC source	Fractional rate constant of digestion (h^{-1})		Indigestible fraction		R^2	
		DM	NDF	DM	NDF	DM	*NDF*
Alfalfa hay	Non-supplemented	0.11 ± 0.027	0.04 ± 0.010^{a}	0.53 ± 0.011^{a}	0.53 ± 0.040^{a}	0.98	*0.81*
	Sucrose	0.08 ± 0.013	0.14 ± 0.030^{b}	0.57 ± 0.011^{b}	0.66 ± 0.020^{b}	0.98	*0.85*
	Starch	0.09 ± 0.026	0.08 ± 0.020^{cd}	0.57 ± 0.016^{b}	0.59 ± 0.030^{a}	0.96	*0.85*
	Sucrose + Starch	0.08 ± 0.013	0.22 ± 0.130^{bd}	0.57 ± 0.011^{b}	0.80 ± 0.020^{c}	0.98	*0.52*
Sugar beet pulp	Non-supplemented	0.09 ± 0.005^{a}	0.07 ± 0.020	0.37 ± 0.004^{a}	0.37 ± 0.040	0.99	*0.83*
	Sucrose	0.08 ± 0.005^{b}	0.06 ± 0.010	0.33 ± 0.008^{b}	0.32 ± 0.040	0.99	*0.87*
	Starch	0.07 ± 0.004^{b}	0.04 ± 0.010	0.34 ± 0.008^{b}	0.36 ± 0.060	0.99	*0.82*
	Sucrose + Starch	0.07 ± 0.002^{b}	0.06 ± 0.010	0.33 ± 0.004^{b}	0.39 ± 0.050	0.99	*0.79*
Wheat bran	Non-supplemented	0.12 ± 0.010^{a}	0.06 ± 0.010^{a}	0.34 ± 0.005^{a}	0.15 ± 0.040^{a}	0.99	*0.91*
	Sucrose	0.15 ± 0.011^{b}	0.06 ± 0.009^{a}	0.35 ± 0.002^{b}	0.08 ± 0.040^{a}	0.99	*0.94*
	Starch	0.11 ± 0.006^{a}	0.12 ± 0.020^{b}	0.34 ± 0.003^{a}	0.32 ± 0.030^{b}	0.99	*0.90*
	Sucrose + Starch	*0.09 ± 0.006^{c}*	*0.12 ± 0.030^{b}*	*0.34 ± 0.006^{ab}*	*0.32 ± 0.040^{b}*	*0.99*	*0.82*

[abc]Means within each column with different letters differ significantly ($P<0.05$)

Table 5: Least square mean of *in vitro* ammonia-N concentration (mg/dl) of alfalfa hay, unmolassed sugar beet pulp and wheat bran supplemented by starch (70 mg/g DM), sucrose (70 mg/g DM) or starch + sucrose (35 mg of each source per g DM)

Feed	NFC source						*P-values*[£]		
	Non	Sucrose	Starch	Sucrose + Starch	Main mean of the feed samples	SEM	Feed effect	NFC effect	*Time effect*
Alfalfa hay	23.5^{a}	20.2^{b}	21.1^{b}	21.0^{b}	21.4^{a}	0.43	<0.01	<0.01	*<0.01*
Sugar beet pulp	17.5	16.5	16.7	16.4	16.8^{b}				
Wheat bran	15.8	14.9	15.4	14.8	15.2^{c}				
Main mean of the NFC source	*18.9^{a}*	*17.2^{b}*	*17.8^{b}*	*17.2^{b}*					

[£]$P<0.01$ for feed source × incubation time, feed source × NFC type × incubation time, NFC type × incubation time, $P=0.206$ for feed source × NFC type. SEM = Standard error of mean. [abc]Means within each row and the column of main mean of the feed samples with different letters differ significantly ($P<0.01$)

Ammonia-N concentration

Ammonia-N concentration of the medium evaluated in the present study is shown in Table 5. A significant effect of supplemental NFC on ammonia-N concentration was detected for alfalfa hay (P<0.01). Time effect and its interactions with feed sources and NFC type were significant (P<0.01). The three-way interaction was also significant (P<0.01). Generally, ammonia-N concentration decreased when alfalfa hay was incubated with SU or ST or SU + ST; however, NFC supplementation had no significant effect on other feeds. The highest concentration of ammonia-N was observed in alfalfa hay, and the lowest one for wheat bran.

Discussion

Supplementation with NFC reduced DM disappearance only in alfalfa hay. Previous *in vitro* results showed that apparent digestibility of DM was not affected when dietary corn starch was partially replaced by sucrose in a total mixed ration using dual-flow continuous-culture fermenters (Vallimont *et al*., 2004). It seems that the effect of NFC on DM disappearance depends on the nature of the basal diet.

Generally, the extent of NDF disappearance was significantly greater in non-supplemented feed sources. In the current experiment, starch had a more negative effect on NDF disappearance than sucrose. We previously found that supplementing pure cellulose with SU or a mixture of SU and ST decreased cellulose disappearance (Rezaii *et al*., 2010). However, Arroquy *et al*. (2005) showed that the types of supplemental NFC did not have a significant effect on NDF disappearance. Previous results indicated some differences among forage sources in their susceptibility to the negative effect of starch on NDF disappearance (Grant, 1994). Reduced rate and extent of NDF digestibility when sucrose was added to various rations were reported (Huhtanen and Khalili, 1991). This effect might be due to the sugar utilizing bacteria competing with the fiber-digesting bacteria for available N, and that the inclusion of adequate quantities of rumen degradable protein (RDP) in the diet might prevent decrease in NDF digestibility (Lee *et al*., 2003). However, higher NDF digestibility with diets including 1 kg/d of sucrose fed twice daily to cows was demonstrated (Huhtanen and Khalili, 1991). Heldt *et al*. (1999b) reported that low levels of supplemental carbohydrate when fed in conjunction with supplemental degradable protein caused NDF digestibility to improve compared with no supplementation. In the present experiment unmolassed sugar beet pulp and wheat bran had more susceptibility than alfalfa hay to adding starch with respect to NDF disappearance (Table 3), this might depend on higher N content in alfalfa hay (Table 1).

Results of the present experiment demonstrated that the fractional rate constant of DM disappearance of unmolassed sugar beet pulp and wheat bran decreased when supplemented with NFC. Starch increased the fractional rate of NDF disappearance in wheat bran, whereas sucrose increased the fractional rate of NDF disappearance in alfalfa hay. Indigestible fractions of DM were significantly increased when NFC was added to alfalfa hay and sucrose was added to wheat bran (P<0.05). In contrast, supplementing with all kinds of NFC decreased the DM indigestible fraction in unmolassed sugar beet pulp. Generally, the indigestible fraction of NDF was greater for treatments receiving supplemental NFC in alfalfa hay and wheat bran. Arroquy *et al*. (2005) reported that the fractional rate of NDF digestion was decreased in response to adding glucose, maltose or starch. In addition, Piwonka and Firkins (1996) reported that the addition of glucose to an *in vitro* system decreased the rate of fiber digestion and particle-associated activity of carboxymethyl cellulose when the pH of the fermentation was maintained above 6.2. Our results indicated the possibility that the NFC source might interact with the basal feed source.

The medium ammonia-N concentration was decreased when alfalfa hay was incubated with NFC. Chamberlain *et al*. (1985) reported a reduction in ruminal ammonia-N concentration due to sucrose supplementation of a grass silage diet and found that sucrose was more effective than starch in reducing ruminal ammonia-N concentration. Vallimont *et al*. (2004) reported that ammonia-N concentration was not affected by replacing starch with sucrose. The results of the current study confirmed the results of previous studies in which sucrose was included in the ration showed a reduction in ruminal ammonia-N concentration (Lee *et al*., 2003). Henning *et al*. (1993) suggested that the lower ammonia-N concentration on the higher energy diet was a result of a greater microbial utilization of dietary nitrogen from an improved energy and nitrogen synchronization. Hoover and Miller Webster (2001) noted reductions in ruminal ammonia-N in nearly all studies in which sugar was added to the diets, suggesting more effective utilization of rapidly available nitrogen. Hristov *et al*. (2005) demonstrated that the provision of readily fermentable energy might decrease the ammonia-N concentration in the rumen through inhibited production of ammonia and enhanced incorporation of pre-formed feed amino acids, or through enhanced uptake of ammonia for microbial protein synthesis.

Previous studies demonstrated that the effect of supplemental NFC on low quality forage utilization depends on the source of supplemental carbohydrate, the amount of supplemental carbohydrate fed, and the amount of supplemental degradable intake protein in conjunction with the carbohydrate. Results of the present experiment suggested that the feed sources might be effective too. Present results demonstrate that the effect of NFC supplementation on NDF disappearance, under *in vitro* condition, vary among alfalfa hay, unmolassed sugar beet pulp and wheat bran. In general, in our study, alfalfa hay showed more susceptibility than unmolassed sugar beet pulp and wheat bran. In addition, under the *in vitro* conditions employed, the types of supplemental NFC examined were different regarding their effects on NDF disappearance, whereby, starch showed a more negative effect than sucrose. Therefore, it is important to consider the interactions among the feed source and the NFC type, as it aids in formulating diets for maximal ruminal DM and NDF disappearance.

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