

## Effects of Non-Fiber Carbohydrate on *In vitro* First Order Kinetic Disappearance of Neutral Detergent Fiber and Neutral Detergent Insoluble Nitrogen of Wheat Bran

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**Abstract:** An *in vitro* experiment was conducted to determine the effect of supplemental Non-Fiber Carbohydrate (NFC) on disappearance kinetics of Neutral Detergent Fiber (NDF) and Neutral Detergent Insoluble Nitrogen (NDIN) of Wheat Bran (WB) by mixed ruminal microorganisms. Non-supplemented or NFC supplemented WB (70 mg NFC g<sup>-1</sup> DM of WB as Sucrose (WBSu) or Starch (WBSt) or a 1:1 mixture of Sucrose + Starch (WBSuSt)) were incubated in bottles containing rumen fluid medium. The samples were incubated for 4, 8, 16 and 48 h at 39°C. After each incubation time, NDF and NDIN disappearance of WB were measured. Data were analyzed as a complete randomized design using a 4×4 factorial arrangement. The 1st factor consisted of supplemental NFC type and the 2nd factor consisted of incubation time. Kinetic disappearance rate of all samples was determined using first order exponential model of  $D_{(t)} = D_{(0)} \cdot \exp(-k_2 \text{ time}) + I$ ;  $D_{(0)}$  is potentially digestible residues;  $D_{(0)}$  is potentially digestible fraction;  $k$  is fractional rate constant of disappearance (h<sup>-1</sup>) and  $I$  is indigestible fraction. In this experiment, Including of NFC in the fermentation medium had a significant ( $p < 0.01$ ) effect on the disappearance of NDF (0.48, 0.49, 0.45 and 0.46, respectively, for WB, WBSu, WBSt, WBSuSt) and NDIN (0.52, 0.58, 0.50 and 0.55, for WB, WBSu, WBSt, WBSuSt, respectively) of wheat bran. The fractional rate constant of NDF disappearance was greater ( $p < 0.05$ ) in both WBSt and WBSuSt samples compared with non-supplemented (0.06, 0.06, 0.12 and 0.12 h<sup>-1</sup>, for WB, WBSu, WBSt, WBSuSt, respectively). Also, indigestible fraction of NDF was greater ( $p < 0.05$ ) for treatments receiving starch or sucrose + starch as the source of supplemental NFC when compared with non-supplemented WB. However, when sucrose was added, the indigestible fraction of WB was similar with the non-supplemented sample. The fractional rate of NDIN disappearance in WB exhibited ( $p < 0.05$ ) an increase in response to adding the sucrose + starch supplement when compared with the non-supplemented WB and it was decreased when starch was added. Including of sucrose in the medium had no significant effect on fractional rate of NDIN disappearance. Indigestible fraction of NDIN was significantly increased when it was supplemented by starch or sucrose + starch ( $p < 0.05$ ).

**Key words:** Non-fiber carbohydrates, wheat bran, disappearance, neutral detergent fiber, neutral detergent insoluble nitrogen

### INTRODUCTION

Carbohydrates comprise approximately 60-70% of the lactating dairy cow's diet. These compounds, in plant cells, served as structural or reservoirs materials. The structural carbohydrates plus lignin, ash and a part of Nitrogen (N) chemically isolated from cell plants and known as Neutral Detergent Fiber (NDF). While, the reservoirs and water soluble carbohydrates are known as Non-Fiber Carbohydrates (NFC). There is some evidence that showed supplementing forage-based diets with feedstuffs that contain high concentrations of rapidly

digestible NFC decreased forage fiber disappearance (Huhtanen and Khalili, 1991; Khalili and Huhtanen, 1991; Heldt *et al.*, 1999). Type of supplemental carbohydrate provided in conjunction with forage has been suggested to be a factor that might impact on NDF disappearance (Fondevila *et al.*, 2002). Different types of NFC have been shown to differ in the yield of microbial crude protein from their fermentation *in vitro* (Hall and Herejk, 2001). *In vivo* work reported by Heldt *et al.* (1999) indicated that supplementation with starch had a more negative effect on forage fiber disappearance than did simple sugars. The method of fractionation, using detergent solutions, made

an ability to divide the nitrogen components into neutral and acid detergent soluble and insoluble fractions. Neutral Detergent Insoluble Nitrogen (NDIN) is the nitrogen contained in the cell wall of the plant. This fraction is determined by measuring the nitrogen content of neutral detergent fiber (Janicki and Stallings, 1987). This nitrogen is not all indigestible, but, is considered more slowly degraded in the rumen. It is also considered as an index to estimate the rumen undegraded protein fraction (Van Soest, 2007). Knowing the distribution of N within any feedstuffs and understanding the ruminal disappearance kinetics of N are important considerations to acquire the greatest benefit from the feeding models (Danesh *et al.*, 2008; Ogden *et al.*, 2006). Although, there is considerable evidence that evaluated the effect of NFC on extend and rate of NDF disappearance. However, there is not any evidence in relation of impact of NFC on disappearance of NDIN.

Therefore, the objective of this study was to determine the effect of supplementing sucrose and/or starch on *in vitro* NDF and NDIN disappearance of wheat bran as non-forage NDF source.

## MATERIALS AND METHODS

**Substrates and medium preparation:** Experimental substrate was wheat bran that was ground using a Willey mill to pass 0.75 mm screen and dried at 80°C for 48 h. The anaerobic cultural technique utilized was that described by Dehority (1969). The fermentation medium was prepared according to that described by Arroquy *et al.* (2005), included 400 mL cell-free ruminal fluid, cellobiose (0.05 g), K<sub>2</sub>HPO<sub>4</sub> (0.45 g), KH<sub>2</sub>PO<sub>4</sub> (0.45 g), NaCl (0.90 g), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.90 g), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.09 g), CaCl<sub>2</sub> (0.09 g), resazurin (0.01 g), NaHCO<sub>3</sub> (4 g) and cysteine-HCl (0.5 g) per liter of medium. Rumen fluid was obtained from 4 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 DM per day, respectively), strained through 4 layers cheesecloth and centrifuged at 3000 RPM for 5 min. Then, the supernatant was centrifuged at 15000 RPM for 15 min. Total 45 mL of medium were distributed into a 100 mL bottle containing each experimental sample and autoclaved in 120°C for 20 min. Then, each bottle was inoculated with 5 mL of cloth-cheese strained rumen fluid and finely bubbled with CO<sub>2</sub>, sealed and incubated. Previous to the inoculation, the rumen 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 non-fiber carbohydrate (sucrose and/or starch) supplemented samples were incubated for 4, 8, 16 and 48 h at 39°C (3 bottles per each treatment per incubation). The supplementation was carried out as 70 mg g<sup>-1</sup> DM of Wheat Bran as Sucrose (WBSu) or Starch (WBSt) or Sucrose + Starch (as 1:1, WBSuSt). The experiment was performed as a completely randomized design using a 4×4 factorial arrangement. The 1st factor consisted of supplemental NFC type: none (i.e., wheat bran only without supplementation), sucrose, starch and sucrose + starch. The 2nd factor consisted of incubation time. After each incubation time, bottle content was filtered through a 22 µm filter paper. Unfiltered samples were analyzed for NDF as described by Van Soest *et al.* (1991). Sodium sulfite was used in the NDF analyses. NDF assayed without a heat stable amylase and expressed including residual ash. Neutral detergent insoluble N were determined by the NDF procedure described previously followed by Kjeldahl N analysis (Kjeltec Auto, 1300, Foss Electric, Copenhagen, Denmark).

**Statistical analysis:** Data were analyzed using GLM procedure of SAS and means were compared using the LSMEANS procedure. Terms in the model were NFC type, incubation time, NFC type x incubation time. The model of statistical analysis for this experiment was:

$$Y_{ij} = \mu + n_i + t_j + (nt)_{ij} + \varepsilon_{ij}$$

where:

$Y_{ik}$  = The dependent variable

$\mu$  = The overall mean

$n_i$  = Fixed effect of NFC type

$t_j$  = Fixed effect of incubation time

$(nt)_{ij}$  = Interaction between NFC type and incubation time

$\varepsilon_{ij}$  = Residual error

A non-linear 1st order model was used to estimate the disappearance kinetic parameters of NDF and NDIN. The model was:

$$D_{(t)} = D_{(0)} \cdot \exp(-k \cdot \text{time}) + I$$

where:

$D_{(0)}$  = Potentially digestible residues

$D_{(t)}$  = Potentially digestible fraction of NDF or NDIN

$k$  = Fractional rate constant of disappearance (h<sup>-1</sup>)

$I$  = Indigestible fraction

## RESULTS AND DISCUSSION

Effect of NFC type on *in vitro* 1st order NDF fractional rate constant and indigestible fraction of wheat

Table 1: Effect of supplemental Non-Fiber Carbohydrate (NFC) on *in vitro* Neutral Detergent Fiber (NDF) disappearance of wheat bran

Time (h)	Treatments*				SEM	p-value		
	WB	WBSu	WBSt	WBSuSt		NFC type effect	Time effect	NFC type x time effect
4	0.39 <sup>a</sup>	0.37 <sup>a</sup>	0.39 <sup>a</sup>	0.47 <sup>b</sup>	0.013	<0.01	<0.01	<0.01
8	0.47	0.45	0.47	0.47	-	-	-	-
16	0.53 <sup>a</sup>	0.57 <sup>a</sup>	0.45 <sup>b</sup>	0.45 <sup>b</sup>	-	-	-	-
48	0.55 <sup>abc</sup>	0.59 <sup>b</sup>	0.50 <sup>c</sup>	0.48 <sup>cd</sup>	-	-	-	-
Total	0.48 <sup>ab</sup>	0.49 <sup>a</sup>	0.45 <sup>c</sup>	0.46 <sup>bc</sup>	-	-	-	-

\*WB = Wheat Bran, WBSu = Wheat Bran + Sucrose, WBSt = Wheat Bran + Starch, WBSuSt = Wheat Bran + Sucrose + Starch, <sup>abc</sup>In each row, means with different letters are significant ( $p < 0.05$ )

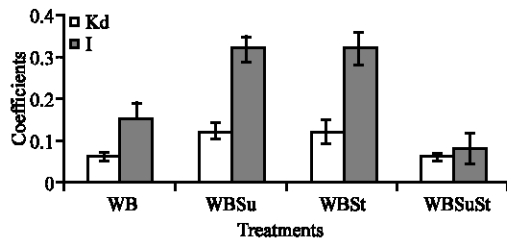


Fig. 1: First order fractional rate constant ( $K_d$ ,  $h^{-1}$ ) and Indigestible fraction (I) of NDF of Wheat Bran (WB), supplemented with Sucrose (WBSu), Starch (WBSt) or Sucrose + Starch (WBSuSt)

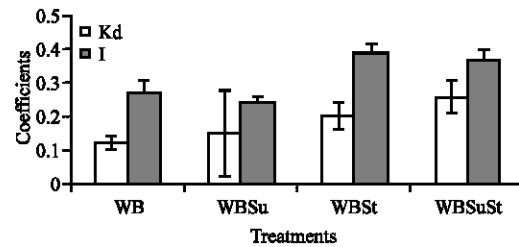


Fig. 2: First order fractional rate constant ( $K_d$ ,  $h^{-1}$ ) and Indigestible fraction (I) of NDIN of Wheat Bran (WB), supplemented with Sucrose (WBSu), Starch (WBSt) or Sucrose + Starch (WBSuSt)

bran is shown in Fig. 1. Fractional rate constant of NDF disappearance in wheat bran increased when starch or sucrose + starch were served as NFC supplement. In addition, indigestible fraction of NDF of wheat bran was significantly increased when it was supplemented by starch or sucrose + starch ( $p < 0.05$ ). NDF disappearance of wheat bran supplemented by sucrose, starch or sucrose + starch is shown in Table 1. NFC type had a significant ( $p < 0.01$ ) effect on NDF disappearance of wheat bran. Also, time effect and interaction between NFC type x incubation time were significant ( $p < 0.01$ ). Generally, in the current experiment starch had more negative effect than sucrose on *in vitro* NDF disappearance of wheat bran, particularly after 16 h incubation. However, sucrose supplementation had no significant effect on NDF disappearance of wheat bran. Results of the *in vitro* study showed that the first order fractional rate of DM digestion of a feed might be influenced by source of NFC (Danesh *et al.*, 2008a). Also, it has been showed that the source of NFC might affect on first order fractional rate of NDF of alfalfa hay (Danesh *et al.*, 2008b). In addition, the results of the current experiment are similar with the observation of *in vivo* research reported by Heldt *et al.* (1999) that supplementation with starch had a more negative effect on forage fiber disappearance than did simple sugars. However, Arroquy *et al.* (2005) in an *in vitro* experiment showed that the types of supplemental NFC (glucose, maltose, corn starch and soluble starch) did not have substantially different effect in regards of NDF disappearance. While, it has been reported that rate

and extent of NDF digestibility reduced when sucrose is added to the ration (Huhtanen and Khalili, 1991; Khalili and Huhtanen, 1991; Heldt *et al.*, 1999), in the present experiment, supplementing with sucrose did not show an effect on rate and extend of NDF disappearance of wheat bran.

Effect of NFC types on *in vitro* 1st order NDIN fractional rate constant of disappearance and indigestible fraction of wheat bran is shown in Fig. 2. Fractional rate constant of NDIN disappearance of wheat bran was significantly influenced by supplemental NFC ( $p < 0.05$ ). In this regards, starch supplementation decreased fractional rate of NDIN disappearance. The fractional rate of NDIN disappearance in wheat bran exhibited ( $p < 0.05$ ) an increase in response to adding sucrose + starch when compared with the non-supplemented samples. In contrast, sucrose supplementation did not have any significant effect on fractional rate constant of NDIN disappearance. Generally, indigestible fraction of NDIN was significantly greater for treatments receiving supplemental NFC when compared with non-supplemented sample (except when sucrose was added). However, when Su was supplemented, indigestible fraction of NDIN was similar with non-supplemented wheat bran. NDIN disappearance of wheat bran supplemented with NFC is shown in Table 2. The effect of NFC type and incubation time and interaction between them were significant ( $p < 0.01$ ). As observed for NDF disappearance, starch had more negative effect on increasing indigestible fraction of NDIN and reduced

Table 2: Effect of supplemental Non-Fiber Carbohydrate (NFC) on *in vitro* Neutral Detergent Insoluble Nitrogen (NDIN) disappearance of wheat bran

Time (h)	Treatments*				SEM	p-value		
	WB	WBSu	WBSt	WBSuSt		NFC type effect	Time effect	NFC type x time effect
4	0.35	0.37	0.38	0.47	0.033	<0.01	<0.01	0.03
8	0.38	0.49	0.44	0.47	-	-	-	-
16	0.66 <sup>abc</sup>	0.74 <sup>b</sup>	0.56 <sup>c</sup>	0.62 <sup>abc</sup>	-	-	-	-
48	0.72	0.75	0.63	0.67	-	-	-	-
Total	0.52 <sup>abc</sup>	0.59 <sup>b</sup>	0.50 <sup>c</sup>	0.55 <sup>abc</sup>	-	-	-	-

\*WB = Wheat Bran, WBSu = Wheat Bran + Sucrose, WBSt = Wheat Bran + Starch, WBSuSt = Wheat bran + Sucrose + Starch, <sup>abc</sup>In each row, means with different letters are significant (p<0.05)

constant rate of NDIN disappearance. The nitrogen associated with NDF is mostly cell wall-bound protein plus other nitrogen compounds and includes indigestible nitrogen found in the acid detergent residue (NRC, 2001). The Nitrogen Insoluble in Neural Detergent solution (NDIN), but, soluble in acid detergent is digestible and consist of slowly degraded protein (Licitra *et al.*, 1996). Krishnamoorthy *et al.* (1982) demonstrated that over 30% of total nitrogen in forages and fermented grains was NDIN. The results of this experiment showed that disappearance of this protein partition might be influenced by the other parts of ration including NFC. In addition, the results of the current experiment demonstrated that type of NFC supplemented could be effective in this regards.

### CONCLUSION

The results of the current study demonstrated that the impact of supplemental non-fiber carbohydrate on wheat bran disappearance of NDF and NDIN depends on the source of supplemental carbohydrate. Under the *in vitro* conditions employed, starch had more negative effect on rate and extent of wheat bran NDF and NDIN disappearance than sucrose. The result of the present study regarding the effect of source of NFC on NDIN disappearance under *in vitro* condition is unique. The finding might be useful to understand the degradation pattern of crude protein sources and doing a better diet formulation for maximal nitrogen efficiency in ruminants.

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