Iranian Journal of Veterinary Research, Shiraz University, Vol. 11, No. 2, Ser. No. 31, 2010

Effect of non-fiber carbohydrates on *in vitro* first order kinetics disappearance of cellulose

Rezaii, F.[1]; Danesh Mesgaran, M.[2*] and Heravi Mousavi, A. R.[2]

[1]Graduated from Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; [2]Department of Animal Sciences, Excellence Center for Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

[*]**Correspondence:** M. Danesh Mesgaran, Department of Animal Sciences, Excellence Center for Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. E-mail: Danesh@um.ac.ir

(Received 14 Jan 2009; revised version 12 Dec 2009; accepted 13 Jan 2010)

Summary

An *in vitro* experiment was conducted to determine the effect of supplemental non-fiber carbohydrate (NFC) on the disappearance kinetics of cellulose (Ce) by mixed ruminal microorganisms. Non-supplemented or NFC supplemented cellulose (467 mg NFC/g cellulose as sucrose (CeSu) or starch (CeSt) or a 1:1 mixture of sucrose + starch (CeSuSt)) were incubated for 24, 48, and 96 h at 39°C. After each incubation time, pH, ammonia-N concentration and cellulose disappearance were measured. The disappearance kinetics rate of all samples was determined using first order exponential model of $D_{(t)} = D_{(i)}.\exp(-k.\ time) + I$; where $D_{(t)}$ is potentially digestible fraction; $D_{(i)}$ is potentially digestible residues; k is digestion rate of cellulose (h^{-1}) and I is indigestible fraction. In this experiment, inclusion of NFC to the rumen fluid medium resulted in a significant depression ($P<0.01$) in the extent of cellulose disappearance. The disappearance rate constant of cellulose was significantly higher ($P<0.05$) in non-supplemented samples as compared with those treatments containing NFC. The indigestible fraction of cellulose was significantly higher ($P<0.05$) for treatments containing sucrose or sucrose + starch as the source of supplemental NFC as compared with non-supplemented cellulose. However, when starch was added, the indigestible fraction of cellulose was similar to those of non-supplemented samples. The inclusion of NFC resulted in a significant reduction ($P<0.01$) in pH of the medium, but had no significant effect ($P>0.05$) on ammonia-N concentration.

Key words: Non-fiber carbohydrates, Cellulose, Disappearance kinetics, Ammonia-N

Introduction

Cellulose is a part of the neutral detergent fiber (NDF) that measures most of the structural components of plants. Other parts of NDF are hemicellulose and lignin (NRC, 2001). Cellulose is the most abundant natural polymer, but mammals do not produce enzymes that can degrade it. Ruminant animals utilize cellulose via a symbiotic relationship with ruminal cellulolytic microorganisms (Russell and Wilson, 1996). Ruminal cellulose digestion is a complex microbial process that involves the adhesion of microbial cells to cellulose, cellulose hydrolysis, and fermentation of the resulting cellodextrins to volatile fatty acid (VFA), methane, and CO_2 (Weimer, 1996). Cereal grains are fermented at a faster rate than cellulose, which provide a greater rate of nutrient release. However, as ruminal fermentation rates increase, ruminal pH often declines. *In vitro* and *in vivo* studies indicated that cellulose digestion can be severely inhibited by even modest declines in ruminal pH (Russell and Wilson, 1996). Weiss *et al*. (1989) observed that cows fed an alfalfa-barley diet digested less acid detergent fiber (ADF) and cellulose than did cows fed alfalfa-corn. The depression in ADF and cellulose digestibility by cows fed alfalfa-barley as compared with cows fed alfalfa-corn was attributed to differences in ruminal pH. The effect of ruminal pH on cellulose digestibility has often been confounded by changes in feed intake or by concentration of fiber in the diet (Russell and Wilson, 1996). Decreased fiber

digestion in an *in vivo* condition is often associated with supplementation of forage diets with readily fermentable carbohydrate sources. Heldt *et al*. (1999) reported a decrease in total tract NDF digestion relative to control diet-fed animals in steers supplemented at 0.3% body weight of DM/d with starch, sucrose, glucose or fructose with low-quality, tallgrass-prairie hay. They suggested that the type of supplemental carbohydrate provided in conjunction with forage was also a factor that might affect the fiber digestion. Similar observation was reported for *in vitro* studies. Fondevila *et al*. (2002) suggested that the addition of carbohydrates such as starch negatively impact *in vitro* microbial fermentation of straw, even at an optimal pH. Therefore, information about how cellulose fermentation is affected by different ruminal environmental conditions is necessary for a better understanding of ruminant performance. The objective of this study was to determine the effect of supplementing sucrose and/or starch on *in vitro* cellulose disappearance, medium pH, and ammonia-N concentration.

Materials and Methods

Substrates and medium preparation

The substrate used in this experiment was commercial pure cellulose (Ce). 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), consisting of 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), $MgSO_4.7H_2O$ (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 the 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/d DM, respectively), strained through 4 layers of cheesecloth, and centrifuged at 3000 RPM for 5 min. The supernatant was then 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 in 120°C for 20 min. Each bottle was inoculated with 5 ml of cheesecloth strained rumen fluid and finely bubbled with CO_2, sealed and incubated. Prior to 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 the inoculum, taking care neither to include the large particles that rose to the top nor that which sedimented in the bottom, was introduced anaerobically into the fermentation bottles.

Experimental procedures

This experiment consisted of one run with three fermentation bottles per treatment per time. Each bottle contained 0.15 g of Ce plus 70 mg of supplemental non-fiber carbohydrate (NFC) as sucrose (CeSu) or starch (CeSt) or a 1:1 mixture of sucrose + starch (CeSuSt) as treatments. The bottles were incubated for 24, 48 and 96 h at 39°C. At each sampling time, the fermentation bottles for that time were removed from the incubator, and the pH of each bottle was recorded directly (691 pH m). The bottle content was filtered through a 22-µm filter paper. Non-filtered samples were analyzed for dry matter (DM) (using air forced oven at 48°C for 48 h). The filtered fermentation fluid was analyzed for ammonia-N concentration using the steam distillation method (Kjeltec Auto 1030 Analyzer tecator, Hoganas, Sweden).

Statistical analysis

The data for medium pH, ammonia-N concentration and cellulose disappearance were statistically analyzed using the GLM procedure of SAS (2003) and means were compared at $p<0.05$. Terms in the model were NFC type, incubation time, and NFC type × incubation time (Arroquy *et al*., 2005). The model of statistical analysis was as shown below:

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

Where,
Y_{ij} = the amount of each observation
μ = the overall mean
n_i = the simple effect of NFC type
t_j = the simple effect of incubation time
$(nt)_{ij}$ = the interaction between NFC type and

incubation time
ε_{ij} = residual error

The disappearance kinetics rate of treatments was determined using a first order exponential model. The model was

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

where
$D_{(t)}$ = potentially digestible fraction
$D_{(i)}$ = potentially digestible residues
k = digestion rate of cellulose (h^{-1})
I = indigestible fraction

Results

A significant NFC type × incubation time interaction (P<0.01) was evident for the extent of cellulose disappearance (Fig. 1). Inclusion of NFC in the fermentation mixture resulted in a significant (P<0.01) depression in the extent of cellulose disappearance as compared with the non-supplemented cellulose. This effect was more obvious for starch supplementation. However, the NFC effect on the extent of cellulose disappearance was most clearly evident at the time periods from 0.0 to 48 h of incubation. The inclusion of NFC in the fermentation mixture resulted in a significant depression in the rate of cellulose disappearance as compared with the non-supplemented sample (Fig. 2). Indigestible fraction of cellulose was greater (P<0.05) for treatments receiving Su or SuSt as the source of supplemental NFC when compared with non-supplemented cellulose (Fig. 2a). However, an indigestible fraction of cellulose was similar in the non-supplemented sample and treatment receiving starch. This observation might be a result of depression in the rate of cellulose disappearance. The fractional rate constant (Fig. 2b) of cellulose disappearance was greater (P<0.05) in the non-supplemented sample compared with those containing NFC. However, the degree of depression in the disappearance rate was dependent on the type of supplemental NFC used. When St or a mixture of Su and St were used as the source of supplemental NFC, the rate of cellulose disappearance was significantly (P<0.05) decreased. In contrast, the addition of Su had no significant effect (P>0.05) on the fractional rate of cellulose disappearance.

The NFC type × incubation time interaction was significant (P<0.01) for the pH of the experimental medium (Fig. 3a). Overall medium pH was significantly higher (P<0.01) for the non-supplemented cellulose as compared with NFC supplemented treatments, particularly after 48 h of incubation. The pH values of the medium averaged across incubation times were 6.32, 6.08, 6.10 and 6.09 for the Ce, CeSu, CeSt

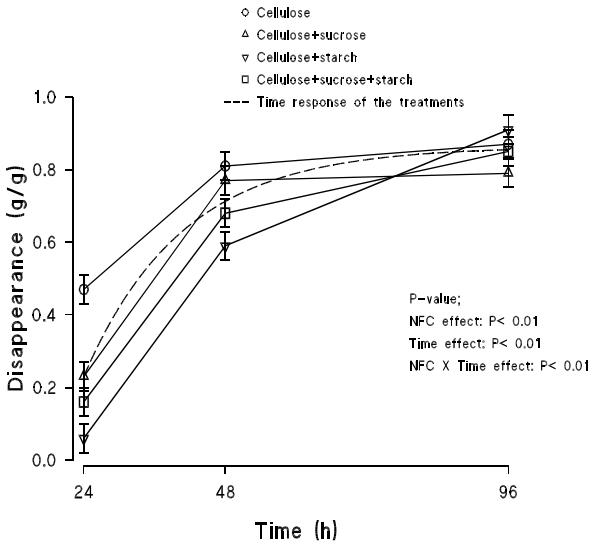

Fig. 1: *In vitro* disappearance (Mean ± SEM) of cellulose, and cellulose supplemented with sucrose, starch or sucrose + starch (1:1)

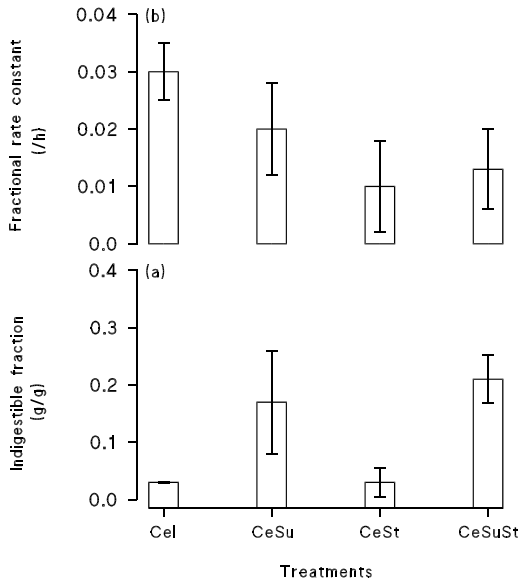

Fig. 2: First order indigestible fraction (a) and fractional rate constant (b) of cellulose (Ce), and cellulose supplemented with sucrose (Su), starch (St) or Su + St as 1:1 (Mean ± SEM)

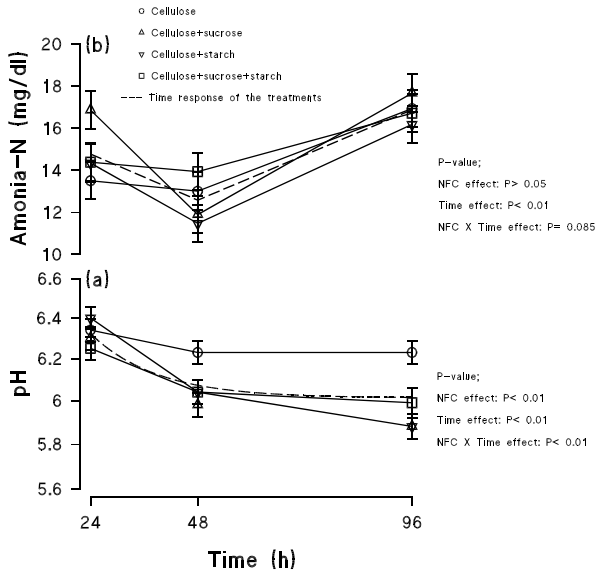

Fig. 3: *In vitro* medium pH (a) and ammonia-N (b) concentration (Mean ± SEM) of cellulose, and cellulose supplemented with sucrose, starch or sucrose + starch (1:1)

and CeSuSt, respectively. The pH of all treatments in the present study declined through the first 24 h and then stabilized. Non-supplemented cellulose stabilized the pH significantly (P<0.01) faster than those treatments which provided supplemental NFC.

The NFC type × incubation time interaction was not significant (P>0.05) for ammonia-N production. Similarly, the inclusion of NFC had no significant (P>0.05) effect on the ammonia-N concentration (Fig. 3b).

Discussion

In this experiment, the inclusion of NFC in the fermentation mixture negatively influenced the extent of cellulose disappearance. This effect was more obvious for starch supplementation. The results of the present study confirmed the observation of Heldt *et al*. (1999) who indicated supplementation with starch had a more negative effect on forage fiber digestion than did simple sugars. However, Arroquy *et al*. (2005), in an *in vitro* experiment, found that the type of supplemental NFC (sugar vs. starch) did not substantially affect the NDF digestion. Miron *et al*. (1990) showed that inclusion of soluble sugars reduced the rate of alfalfa hay cell wall degradation by a mixed rumen population without reducing the extent of the overall cell wall disappearance. These researchers observed that the ability of cellulolytic bacteria to attach to cellulose was higher when adapted to an alfalfa hay cell wall vs. cellobiose, indicating the possible involvement of these materials in the attachment mechanism of the bacteria. This effect of sugar and, presumably, some other forms of NFC on the colonization of fiber by cellulolytic bacteria may be important in cellulose disappearance.

The results of the present study indicated that the fractional rate of cellulose disappearance (Fig. 2b) was significantly greater (P<0.05) in the non-supplemented sample as compared with those treatments containing NFC. This effect was more obvious when St or SuSt were used as the source of supplemental NFC. Previous studies showed that the source of NFC might affect the first order fractional rate of NDF of alfalfa hay (Danesh Mesgaran *et al*., 2008). Arroquy *et al*. (2005) observed that the adverse effect of supplemental NFC was most readily evident in the form of a depression in the fractional rate of disappearance, which was largely independent of pH adjustments. In their experiment, NFC was able to depress the disappearance rate even when ruminal pH was maintained in a seemingly desirable range (>6.2), and it was concluded that both the presence of readily available NFC and medium pH might affect the NDF digestion. Similar finding were reported by Piwonka and Firkins (1996), who found that the addition of glucose to an *in vitro* system decreased the rate of fiber disappearance and particle-associated activity of carboxymethyl cellulase when the pH of the fermentation was maintained above 6.2.

Hiltner and Dehority (1983) investigated the rate of cellulose disappearance in the presence of either glucose or cellobiose for the three predominant species of cellulolytic rumen bacteria (i.e. *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Bacteroides succinogenes*). In their experiment, the lag phase of cellulose disappearance was shortened when a soluble carbohydrate was added and the rate of cellulose disappearance slowed markedly for *B.*

succinogenes and *R. flavefaciens*, slowed less for *R. albus* after the cellobiose or glucose had been utilized, and was accompanied by a decrease in pH. Such conditions might be involved in the changes in the rate of cellulose disappearance which were observed in our experiments. Furthermore, several studies have reported a reduced rate and extent of NDF digestion when sucrose was added to the ration (Huhtanen and Khalili, 1991; Khalili and Huhtanen, 1991; Heldt *et al*., 1999). This effect might be due to the NFC-fermenting bacteria competing with the fiber-digesting bacteria for available N, and that the inclusion of adequate quantities of rumen degradable protein in the diet might prevent Su from decreasing NDF digestibility (Lee *et al*., 2003). Therefore, this effect might be important in the depression of cellulose disappearance observed in the present study as well.

In this experiment, medium pH was significantly higher ($P<0.01$) for the non-supplemented cellulose as compared with NFC supplemented treatments. Vallimont *et al*. (2004) reported that replacing dietary St with Su did not affect ruminal pH and the average value for ruminal pH was 5.97. Sannes *et al*. (2002) reported that ruminal pH was not affected by adding 3.2% of Su in the diet and averaged 6.02. Khalili and Huhtanen (1991) and Lee *et al*. (2003) reported a significant effect of Su, included in the diet, on ruminal pH. Lee *et al*. (2003) found a linear reduction in ruminal pH from 6.4 to 6.0 as sugar infusion levels were increased. In their experiment, the lowest pH was observed at the highest level of water soluble carbohydrate inclusion. This decline in ruminal pH probably occurred as a result of the rapid fermentation of water soluble carbohydrate, resulting in a rapid increase in VFA and lactic acid concentrations (Obara *et al*., 1991). In the present study, the reduction in pH, associated with NFC inclusion, was accompanied by a reduction in cellulose disappearance, which supported the findings of Grant and Mertens (1992), which showed an inhibition of cellulolytic, hemicellulolytic and pectinolytic organisms at low pH.

The results of the present study showed that the inclusion of NFC had no significant effect ($P>0.05$) on medium ammonia-N concentration. This result is similar to the reports of Vallimont *et al*. (2004) who observed that replacing St with Su had no effect on ammonia-N concentration. However, Rezaii *et al*. (2008) indicated that ammonia-N concentration was lower when steers were fed Su or St than only the basal diet. Hristov *et al*. (2005) demonstrated that water soluble sugars resulted in a decrease in ammonia-N concentration in the rumen, through decreased ammonia production; while starch increased the uptake of ammonia for microbial protein synthesis. Chamberlain *et al*. (1985) reported a reduction in ruminal ammonia-N concentration due to Su supplementation of a grass silage diet and found that Su was more effective than St in reducing ruminal ammonia-N concentration. Vallimont *et al*. (2004) pointed out that results from *in vivo* and *in vitro* experiments might differ because of the contribution of rumen recycled nitrogen.

In vitro supplementation of cellulose with NFC had the potential to depress the rate and extent of cellulose disappearance. However, the degree of depression was dependent on the type of supplemental NFC used. In the present study, St had a greater negative effect on the rate and extent of Ce disappearance than Su. Inclusion of supplemental NFC, regardless of NFC type, declined pH as compared with non-supplemented cellulose. The effect of supplemental NFC on *in vitro* plant cell wall component degradation, and medium pH and ammonia-N concentration should be considered using different rumen digestible protein.

References

Arroquy, JI; Cochran, RC; Nagaraja, TG; Titgemeyer, EC and Johnson, DE (2005). Effect of types of non-fiber carbohydrate on *in vitro* forage fiber disappearance of low-quality grass hay. Anim. Feed Sci. Technol., 120: 93-106.

Chamberlain, DG; Thomas, PC; Wilson, W; Newbold, CJ and MacDonald, JC (1985). The effects of carbohydrate supplements on ruminal concentrations of ammonia in animals given diets of grass silage. J. Agric. Sci., 104: 331-340.

Danesh Mesgaran, M; Rezaii, F and Heravi Mousavi, AR (2008). The effect of non-fiber carbohydrate on *in vitro* first order NDF disappearance of alfalfa. J. Dairy Sci., 91: 244.

Dehority, BA (1969). Pectin-fermenting bacteria isolated from the bovine rumen. J. Bacteriol., 99: 189-196.

Fondevila, M; Barrios-Urdaneta, A; Balcells, J and Castrillo, C (2002). Gas production from straw incubated *in vitro* with different levels of purified carbohydrates. Anim. Feed Sci. Technol., 101: 1-15.

Grant, RJ and Mertens, DR (1992). Development of buffer systems for pH control and evaluation of pH effects on fiber digestion *in vitro*. J. Dairy Sci., 75: 1581-1587.

Heldt, JS; Cochran, RC; Stokka, GL; Farmer, CG; Mathis, CP; Titgemeyer, EC and Nagaraja, TG (1999). Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. J. Anim. Sci., 77: 2793-2802.

Hiltner, P and Dehority, BA (1983). Effect of soluble carbohydrates on disappearance of cellulose by pure cultures of rumen bacteria. Appl. Environ. Microbiol., 46: 642-648.

Hristov, AN; Ropp, JK; Grandeen, KL; Abedi, S; Etter, RP; Melgar, A and Foley, AE (2005). Effect of carbohydrate source on ammonia utilization in lactating dairy cows. J. Anim. Sci., 83: 408-421.

Huhtanen, P and Khalili, H (1991). Sucrose supplements in cattle given grass silage-based diet. 3. Rumen pool size and digestion kinetics. Anim. Feed Sci. Technol., 33: 275-287.

Khalili, H and Huhtanen, P (1991). Sucrose supplements in cattle given grass silage-based diet. 2. Digestion of cell wall carbohydrates. Anim. Feed Sci. Technol., 33: 263-273.

Lee, MRF; Merry, RJ; Davies, DR; Moorby, JM; Humphreys, MO; Theodorou, MK; MacRae, JC and Scollan, ND (2003). Effect of increasing availability of water-soluble carbohydrates on *in vitro* rumen fermentation. Anim. Feed Sci. Technol., 104: 59-70.

Miron, J; Ben-Ghedalia, D; Yokoyama, MT and Lamed, R (1990). Some aspects of cellobiose effect on bacterial cell surface structures involved in lucerne cell walls utilization by fresh isolates of rumen bacteria. Anim. Feed Sci. Technol., 30: 107-120.

NRC (2001). *Nutrient requirements of dairy cattle*. 7th Rev. Edn., Chap. 4, Washington, D.C., National Academy Press. PP: 34-42.

Obara, Y; Dellow, DW and Nolan, JV (1991). The influence of energy rich supplements on nitrogen kinetics in ruminants. In: Physiological aspects of disappearance and metabolism in ruminants. San Diego, Academic Press. PP: 515-539.

Piwonka, EJ and Firkins, JL (1996). Effect of glucose fermentation on fiber digestion by ruminal microorganisms *in vitro*. J. Dairy Sci., 79: 2196-2206.

Rezaii, F; Danesh Mesgaran, M; Heravi Moosavi, AR and Nasiri, MR (2008). Effect of supplemented diet by sucrose or starch on ruminal pH and ammonia-N concentration in Holstein steers. *Proceedings of British Society of Animal Science*. P: 189.

Russell, JB and Wilson, DB (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci., 79: 1503-1509.

Sannes, RA; Messman, MA and Vagnoni, DB (2002). Form of rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. J. Dairy Sci., 85: 900-908.

SAS (2003). *SAS User's Guide: Statistics*. Version 9.1 Edn., SAS Institute, Cary, NC.

Vallimont, JE; Bargo, F; Cassidy, TW; Luchini, ND; Broderick, GA and Varga, GA (2004). Effects of replacing dietary starch with sucrose on ruminal fermentation and nitrogen metabolism in continuous culture. J. Dairy Sci., 87: 4221-4229.

Weimer, PJ (1996). Why don't ruminal bacteria digest cellulose faster? J. Dairy Sci., 79: 1496-1502.

Weiss, WP; Fisher, GR and Erickson, GM (1989). Effect of source of neutral detergent fiber and starch on nutrient utilization by dairy cows. J. Dairy Sci., 72: 2308-2315.