Short communication: Difructose anhydride III promotes calcium absorption from the duodenum in cattle

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ABSTRACT

Difructose anhydride (DFA) III promotes the intestinal absorption of calcium via a paracellular pathway in rats. In dairy cows, DFA III reaches the duodenum without being degraded by ruminal bacteria and hence could be used to control hypocalcemia. The aims of the present study were to investigate the percentage of DFA III that appears in the duodenum of cows and to determine the effect of DFA III on calcium absorption from duodenal fluid. The first experiment was performed in 3 ruminally and duodenally cannulated dry Holstein cows in a 3 × 3 Latin square design. Each experimental period lasted 7 d. On the first day, the cows were ruminally fed one of the following treatments: 0 (DFA0), 50 (DFA50), or 100 (DFA100) g/d of DFA III, using cobalt-EDTA as a liquid phase marker. Difructose anhydride III was detected in duodenal fluid 1 h after feeding, and its concentration peaked 4 h after feeding, in a dose-dependent manner. The percentages of DFA III that appeared in the duodenum after the DFA50 and DFA100 treatments were $69.1 \pm 7.0\%$ and $67.9 \pm 5.6\%$, respectively. The second experiment used the everted duodenal sacs of cattle ($n = 7$ in each group). Sacs were incubated in artificial mucosal fluid containing 1 mM DFA III or no DFA III (control) for 60 min with 100% O₂ in a water bath at 37°C. After incubation, the calcium concentration of the artificial serosal fluid in the everted sacs was measured. Calcium absorption was higher in the DFA III-treated group than in the control group (803 ± 161 and 456 ± 74 nmol/cm of sac, respectively). The above results demonstrate that approximately 70% of administered DFA III reached the duodenum of cows intact. Moreover, similar to its effects on calcium absorption in rats, DFA

III promoted calcium absorption via a paracellular pathway in the duodenum of cows.

Key words: difructose anhydride (DFA) III, calcium, duodenum

Short Communication

Transepithelial calcium transport in the intestine occurs via 2 routes, a transcellular pathway and a paracellular pathway (Bronner, 1998). Transcellular absorption is dependent on an active transport process driven by metabolic energy. The transport of Ca ions across the cytoplasm is the rate-limiting step because the process depends on a Ca-binding protein (calbindin-D9k, CaBP9k; Bronner, 1998). This protein is localized in the upper duodenum and is completely dependent on the presence of 1,25-dihydroxyvitamin D. Paracellular Ca absorption involves passive transport (diffusion) and requires a Ca concentration gradient between the lumen and the basolateral side of the intestinal mucosa. Evidence has accumulated that tight junctions, located on the luminal side of adjacent epithelial cells, regulate the absorption of various nutrients, including Ca (Ballard et al., 1995). In monogastric animals, transepithelial Ca-transport activity occurs not only in the duodenum, jejunum, and ileum but also in the cecum, which is the site with the highest Ca-binding protein activity (Perret et al., 1985; Howard et al., 1993). In contrast, in dairy cows, the highest transepithelial Ca-transport activity is believed to occur in the proximal region of the small intestine because detectable levels of mRNA coding for intestinal calbindin-D9k are localized only in the duodenum (Yamagishi et al., 2002). Thus, dairy cows are vulnerable to hypocalcemia.

Difructose anhydride (DFA) III (di-D-fructofuranose-1,2':2,3'-dianhydride; Figure 1) is an indigestible disaccharide that can be produced in large quantities from inulin using inulase II from *Arthrobacter* sp. H65-7 (Saito and Tomita, 2000). Difructose anhydride III has been found to enhance intestinal Ca absorption via a paracellular pathway in rats (Suzuki et al., 1998; Mineo

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et al., 2002; Mitamura and Hara, 2005) and humans (Shigematsu et al., 2004; Suzuki and Hara, 2004). One of the major mechanisms was mediated by claudin-1, a component protein of tight junctions that decreases transepithelial electrical resistance (TEER) in the small intestine (Suzuki and Hara, 2006). In cows, ruminal microorganisms have difficulty digesting DFA III (Sato et al., 2006); therefore, intact DFA III reaches the duodenum 1 h after oral ingestion (Nakai et al., 2007). Furthermore, by supplementing the diet of parturient cows with DFA III during the prepartum period (i.e., 3 wk before parturition), hypocalcemia can be controlled (Sato et al., 2007). These reports suggest that DFA III likely reaches the duodenum in cows, as it does in rats, and promotes Ca absorption through a paracellular pathway. However, it is not clear how much DFA III arrives at the duodenum in cows or whether DFA III does indeed promote Ca absorption through a paracellular pathway in the duodenum of cows as it does in rats and humans. Therefore, the aims of the present study were to investigate the percentage of DFA III that reaches the duodenum of cows and to determine the effect of DFA III on Ca absorption from duodenal fluid.

Experiment (**Exp.**) 1 was performed in 3 ruminally and duodenally cannulated dry Holstein cows (673 ± 15 kg of BW and 7.2 ± 1.2 yr of age) in a 3×3 Latin square design. Each experimental period lasted 7 d, with a 3-d sampling period and a 4-d washout period. Cows were housed in individual tiestalls and fed 6.5 kg DM of corn silage, 4.5 kg DM of grass hay, and 1.7 kg DM of commercial concentrate (Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan) once daily at 1000 h. Cows were fed DFA III (Nippon Beet Sugar Mfg. Co. Ltd., Tokyo, Japan) via the ruminal cannula as 100 g of sucrose dissolved in 500 mL of water (**DFA0**), 50 g of DFA III and 50 g of sucrose dissolved in 500 mL of water (**DFA50**), or 100 g of DFA III dissolved in 500 mL of water (**DFA100**) at 1000 h on the first day of each experimental period. Each solution con-

tained 16 g of cobalt-EDTA·4H₂O (containing 2 g of Co) added as liquid phase marker (Udén et al., 1980). Ruminal fluid, duodenal fluid, and feces were collected before (0 h) and at 1, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, and 72 h after DFA III feeding on the first day of each experimental period. Ruminal fluid was squeezed through 4 layers of cheesecloth, and 1 mL of 6 M hydrochloric acid per 100 mL of ruminal fluid was added to the ruminal samples, which were then frozen at -20°C until Co analysis. Duodenal fluids and feces were immediately frozen at -20°C until DFA III and Co concentrations were analyzed. Concentrations of DFA III were analyzed using HPLC as described by Minamida et al. (2006) and Nakai et al. (2007). The detection limit of this method of analysis is 10 $\mu\text{g/mL}$. Concentrations of Co were analyzed using inductively coupled plasma spectroscopy (ICPE-9000; Shimadzu Corp., Kyoto, Japan). To pretreat the samples, the ruminal and duodenal fluids were left untouched, and feces were dried in a forced-air oven at 60°C for 24 h and ground using a Wiley mill (SM2000; Retsch, Haan, Germany). The concentrations of Co at 0 h in the ruminal fluid, duodenal fluid, and feces were subtracted from the concentration at each sampling time. The rate of passage of liquid from the rumen was estimated using the following formula (Yang et al., 2001). The ruminal fluid volume was calculated by dividing the Co dose in the rumen at the 0-h intercept of the fitted equation (**C0**) and then multiplying by the rate of passage of liquid from the rumen to determine the liquid flow rate (Koenig et al., 2002). The appearance of DFA III in the duodenal fluid and feces as a percentage of the dose was calculated for each animal using the following formulas (modified from Ray et al., 2013):

Duodenal (facial) appearance of DFA III as a percentage of dose (%) = [fed Co (g)/recovery of Co in duodenal fluids (feces) (g) $\times$ recovery of DFA III in duodenal fluids (feces) (g)/fed DFA III (g)] $\times$ 100,

Recovery of DFA III or Co in duodenal fluids (g) = $(1/1,000) \times \Sigma[\text{collected duodenal fluid volume (mL)} \times \text{DFA III or Co concentration in duodenal fluid (mM)/molecular weight/1,000}]$,

Recovery of DFA III or Co in feces (g) = $\Sigma[\text{collected feces volume (g)} \times \text{DFA III or Co concentration in feces (mmol/g)/molecular weight/1,000}]$.

Figure 1. Structural formula of difructose anhydride III produced from inulin with *Arthrobacter* sp. H65-7 inulase II (EC 2.4.1.93).

The second experiment (Exp. 2) was performed as described by Kozakai et al. (1999). Seven animals [Holstein ($n = 6$) and Japanese Black ($n = 1$), 30 ± 14 mo of age; animals were selected based solely on availability] were euthanized. Two segments, 10 cm in length, were dissected from the duodenum. The intestinal segments were exfoliated mesentery tissue, and they were everted using a blunt plastic rod. Each sac was instilled with 10 mL of serous fluid (pH 7.4, 20 mM HEPES, 125 mM NaCl, 4 mM KCl, and 10 mM glucose, in 100% O₂ at 37°C) and ligated as both ends with surgical cotton thread. The sacs were transferred to individual flasks containing gassed and warmed artificial mucosal fluid [pH 6.5, 20 mM HEPES, 125 mM NaCl, 4 mM KCl, 10 mM glucose, and 10 mM calcium chloride dehydrate (CaCl₂·2H₂O)]. Two treatments were used: artificial mucosal fluid with 1 mM DFA III and artificial mucosal fluid containing no DFA III (control). Sacs were incubated for 60 min in 100% O₂ in a 37°C water bath. After incubation, the Ca concentration of the artificial serosal fluid in the everted sacs was assayed using a commercial kit (Calcium-E test, Wako Pure Chemical Industries, Osaka, Japan). The inter- and intraassay coefficients of variation were 1.04 and 0.35%, respectively.

All statistical analyses were performed using R 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). In Exp. 1, ruminal volume, ruminal outflow, rate of liquid passage from the rumen, and percentage of DFA III that appeared in the duodenum and feces were fitted to a mixed-effects model and analyzed using the lmerTest package. The fixed effect was treatment, and the random effects were cow and period. Means were compared using the Tukey test. Concentrations of DFA III and Co in the duodenal fluids and feces were analyzed using Pearson correlation coefficients. In Exp. 2, transepithelial Ca absorption (nmol/cm) was analyzed using a paired *t*-test. Differences were considered to be statistically significant when $P < 0.05$.

In Exp. 1, ruminally fed DFA III was detected in the duodenal fluid 1 h after feeding (Figure 2c), consistent with the results of a previous study (Nakai et al., 2007), and the concentration of DFA III peaked 4 h after feeding, in a dose-dependent manner. This finding suggests that DFA III reaches the duodenum intact and that DFA III is resistant to decomposition by rumen microbes (Sato et al., 2006) because they lack highly specific enzymes that are needed to hydrolyze DFA III (Sakurai et al., 1997). Moreover, DFA III immediately dissolved in the rumen liquid phase and outflowed from the rumen to the duodenum, as determined by a strong positive correlation between the concentrations of DFA III and Co (DFA50: $r = 0.91$, $P < 0.01$, DFA100: $r = 0.93$, $P < 0.01$), which was used as a marker of the liquid

phase in duodenal fluid. The average rate of the liquid flow out of the rumen was 10.1%/h (Table 1), which is in the same range as in previous reports (Yang et al., 2001; Koenig et al., 2002). The percentage of DFA III that appeared in the duodenum was $69.1 \pm 7.0\%$ and $67.9 \pm 5.6\%$ for the DFA50 and DFA 100 groups, respectively (means $\pm$ SE; Table 1). Based on this result, we hypothesized that DFA III may be able to promote Ca absorption via a paracellular pathway in the duodenum of cows, similar to its effects on Ca absorption in humans and rats. Meanwhile, the percentages of DFA III that appeared in feces in Exp. 1 were $8.7 \pm 2.2\%$ and $15.3 \pm 4.5\%$ for DFA50 and DFA 100 groups, respectively (Table 1). These results suggest that DFA III was degraded in the large intestine by one or more microbial species. In rats, DFA III has been shown to be degraded by *Ruminococcus productus* in the cecum (Minamida et al., 2005), and organic acids produced by DFA III fermentation in the cecum increase Ca solubility and enlarge the area over which absorption occurs (Suzuki et al., 1998). However, the present study did not consider whether DFA III was microbially degraded and promoted Ca absorption in the large intestine in cows or whether DFA III degradability in the rumen was affected by long-term feeding. These considerations should be addressed in future studies.

In Exp. 2, the amount of Ca absorption by everted sacs that had been prepared from duodenal tissue was higher in the DFA group than in the control group (803 ± 161 and 456 ± 74 nmol/cm of sac, respectively, $P < 0.05$; Figure 3). These results suggest that DFA III promoted duodenal Ca absorption in cows, as we hypothesized. Absorption of Ca was significantly higher (176%) when the sacs contained 1 mM DFA III in artificial mucosal fluid than in the control sacs without DFA III. In this study, the differences in Ca absorption via the transcellular pathway in both treatments were not considered. Because the Ca concentration in the artificial mucosal fluid used in this experiment was 10 mM, which is higher than the Michaelis constant for the saturable transfer of Ca in the everted segments (0.35 mM; Bronner, 1987), the increases in Ca absorption found in this study likely involved changes in the passive paracellular diffusion of Ca in the intestinal mucosa (Kishi et al., 1996).

Suzuki et al. (1998) and Mineo et al. (2002) have also previously reported that higher DFA III concentrations further increase Ca absorption in rats. The DFA50 and DFA100 treatments increased Ca absorption more in Exp. 1 than in Exp. 2. This conclusion is based on the DFA III concentrations, which exceeded 1 mM, in the duodenal fluid from 2 to 6 h after feeding in the DFA50 treatment group and from 2 to 12 h after feeding in the DFA100 treatment group. The highest DFA III con-

Figure 2. Diffructose anhydride (DFA) III and cobalt (Co) concentrations in ruminal and duodenal fluid and feces of cows after being fed 0 g (DFA0, ▲; n = 3), 50 g (DFA50, ○; n = 3), or 100 g (DFA100, □; n = 3) of DFA III. (a) Co concentration in ruminal fluid; (b) DFA III concentration in duodenal fluid; (c) Co concentration in duodenal fluid; (d) DFA III concentration in feces; (e) Co concentration in feces. The vertical bars represent standard errors of the means.

Figure 3. Calcium absorption by everted sacs made from the duodenum (means $\pm$ SEM; nmol/cm of sac). DFA III = 1 mM of difructose anhydride III in artificial mucosal fluid. Letters a and b indicate statistically significant differences ($P < 0.05$) between the results of the treatments.

concentrations in both groups occurred 4 h after feeding and were 1.5 mM in the DFA50 group and 2.2 mM in DFA100 group (Figure 2c). These data suggest that an

effective dose of DFA III reached the duodenum intact, but future studies are needed to quantitatively analyze and verify the correlation between DFA III concentration and Ca absorption rate in cows.

Most cows experience varying degrees of hypocalcemia after parturition. One of the causes of hypocalcemia in older cows is a reduced ability to absorb Ca because the number of vitamin D receptors in intestinal epithelial cells declines with age (Horst et al., 1990). Calcium absorption through the paracellular pathway, which is promoted by DFA III, occurs via passive diffusion mediated by a concentration gradient and is independent of vitamin D receptors within intestinal epithelial cells. Thus, DFA III holds promise for universally preventing hypocalcemia by ensuring sufficient Ca absorption, even in older cows that are vulnerable to this condition because of the reduced number of vitamin D receptors in their duodenums.

In conclusion, approximately 70% of DFA III reached the duodenum of cows intact. Moreover, DFA III promoted Ca absorption via a paracellular pathway in the duodenum, similar to its activity in rats. This Ca absorption route holds promise for decreasing the incidence of hypocalcemia in older cows, which are vulnerable to hypocalcemia because they have decreased numbers of vitamin D receptors in their duodenums.

Table 1. Ruminal passage and percentage of difructose anhydride III (DFA III) in cows

Item	Treatment ¹		
	DFA0	DFA50	DFA100
Intake			
Co, g	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0
DFA III, g	0.0 $\pm$ 0.0	50.0 $\pm$ 0.0	100.0 $\pm$ 0.0
Rumen			
CO, ² mM	4.4 $\pm$ 0.5	4.4 $\pm$ 0.4	4.2 $\pm$ 0.4
Ruminal fluid volume, ³ L	80.5 $\pm$ 10.7	78.1 $\pm$ 7.8	81.7 $\pm$ 8.0
Ruminal flow rate, ⁴ L/h	7.6 $\pm$ 0.8	8.1 $\pm$ 0.6	8.4 $\pm$ 1.0
Rate of passage from the rumen			
Liquid passage, ⁴ %/h	9.6 $\pm$ 0.7	10.4 $\pm$ 0.2	10.2 $\pm$ 0.3
Duodenum			
Co recovery, mg	10.0 $\pm$ 0.9	10.7 $\pm$ 1.8	10.4 $\pm$ 1.1
DFA III recovery, mg	0.0 $\pm$ 0.0	192.5 $\pm$ 45.9	351.7 $\pm$ 25.4
DFA III appearance, ⁵ %	0.0 $\pm$ 0.0 ^a	69.1 $\pm$ 7.0 ^b	67.9 $\pm$ 5.6 ^b
Feces			
Co recovery, mg	160.8 $\pm$ 2.6	175.5 $\pm$ 8.3	158.9 $\pm$ 7.9
DFA III recovery, mg	0.0 $\pm$ 0.0	370.7 $\pm$ 77.4	601.6 $\pm$ 170.7
DFA III appearance, ⁵ %	0.0 $\pm$ 0.0 ^a	8.7 $\pm$ 2.2 ^{ab}	15.3 $\pm$ 4.5 ^b

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

¹Cows were fed DFA III (Nippon Beet Sugar Mfg. Co. Ltd., Tokyo, Japan) via the ruminal cannula as 100 g of sucrose dissolved in 500 mL of water (DFA0; control), 50 g of DFA III and 50 g of sucrose dissolved in 500 mL of water (DFA50), or 100 g of DFA III dissolved in 500 mL of water (DFA100) at 1000 h on the first day of each experimental period.

²CO = Co concentration in the rumen at 0 h after Co feeding (Yang et al., 2001).

³Calculated by dividing the Co dose by CO (Koenig et al., 2002).

⁴Estimated by multiplying by the rate of passage of liquid from the rumen and the ruminal fluid volume (Koenig et al., 2002).

⁵Calculated using a formula modified from Ray et al. (2013).

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REFERENCES

- Ballard, S. T., J. H. Hunter, and A. E. Taylor. 1995. Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. *Annu. Rev. Nutr.* 15:35–55.
- Bronner, F. 1987. Calcium absorption, Pages 1419–1435 in *Physiology of the Gastrointestinal Tract*. 2nd ed. L. R. Johnson, ed. Raven, New York, NY.
- Bronner, F. 1998. Calcium absorption—A paradigm for mineral absorption. *J. Nutr.* 128:917–920.
- Horst, R. L., J. P. Goff, and T. A. Rainhardt. 1990. Advancing age results in reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. *Endocrinology* 126:1053–1057.
- Howard, A., S. Legon, and J. R. F. Walters. 1993. Human and rat intestinal plasma membrane calcium pump isoforms. *Am. J. Physiol.* 265:G917–G925.
- Kishi, K., T. Goda, and S. Takase. 1996. Maltitol increase transepithelial diffusional transfer of calcium in rat ileum. *Life Sci.* 59:1133–1140.
- Koenig, K. M., L. M. Rode, C. D. Knight, and M. Vázquez-Añón. 2002. Rumen degradation and availability of various amounts of liquid methionine hydroxy analog in lactating dairy cows. *J. Dairy Sci.* 85:930–938.
- Kozakai, T., K. Katoh, and Y. Sasaki. 1999. Effect of mucosal ions on calcium absorption in the jejuno-ileum of the goat in vitro. *Anim. Sci. J.* 70:526–534.
- Minamida, K., C. Asakawa, I. N. Sujaya, M. Kaneko, A. Abe, T. Sone, H. Hara, K. Asano, and F. Tomita. 2006. Effects of long-term ingestion of difructose anhydride III (DFA III) on intestinal bacteria and bile acid metabolism in humans. *J. Biosci. Bioeng.* 101:149–156.
- Minamida, K., M. Kaneko, M. Ohashi, I. N. Sujaya, T. Sone, M. Wada, A. Yokota, H. Hara, K. Asano, and F. Tomita. 2005. Effect of difructose anhydride III (DFA III) administration on bile acid and growth of DFA III-assimilating bacterium *Ruminococcus productus* on rat intestine. *J. Biosci. Bioeng.* 99:548–554.
- Mineo, H., H. Hara, N. Shigematsu, Y. Okuhara, and F. Tomita. 2002. Melibiose, difructose anhydride III and difructose anhydride IV enhance net calcium absorption in rat small and large intestinal epithelium by increasing the passage of tight junctions in vitro. *J. Nutr.* 132:3394–3399.
- Mitamura, R., and H. Hara. 2005. Prolonged feeding of difructose anhydride III increases strength and mineral concentrations of the femur in ovariectomized rats. *Br. J. Nutr.* 94:268–274.
- Nakai, T., S. Murata, H. Kikuchi, T. Sato, H. Sadoya, M. Ohtani, M. Hanada, and M. Okamoto. 2007. Detection of dietary difructose anhydride III in the duodenal digesta of steers. *Nihon Chikusan Gakkaiho* 78:57–61. (In Japanese with an English abstract).
- Perret, C., C. Desplan, and M. Thomasset. 1985. Cholecalciferol (a 9-Da cholecalciferol-induced calcium-binding protein) messenger RNA: Distribution and induction by calcitriol in the rat digestive tract. *Eur. J. Biochem.* 150:211–217.
- Ray, P. P., J. Jarrett, and K. F. Knowlton. 2013. Effect of dietary phytate on phosphorus digestibility in dairy cows. *J. Dairy Sci.* 96:1156–1163.
- Saito, K., and F. Tomita. 2000. Diffructose anhydrides: Their mass-production and physiological function. *Biosci. Biotechnol. Biochem.* 64:1321–1327.
- Sakurai, H., A. Yokota, Y. Sumita, Y. Mori, H. Matsui, and F. Tomita. 1997. Metabolism of DFA III by *Arthrobacter* sp. H65-7: Purification and properties of a DFA III hydrolysis enzyme (DFA IIIase). *Biosci. Biotechnol. Biochem.* 64:989–993.
- Sato, T., H. Kikuchi, T. Nakai, H. Sadoya, M. Hanada, and M. Okamoto. 2006. Effect of ruminal bacteria on degradability of difructose anhydride III. *Nihon Chikusan Gakkaiho* 77:395–399. (In Japanese with an English abstract).
- Sato, T., T. Nakai, H. Sadoya, M. Ohtani, M. Hanada, and M. Okamoto. 2007. Effect of difructose anhydride III on hypocalcemia in dairy cows after calving. *Nihon Chikusan Gakkaiho* 78:37–43. (In Japanese with an English abstract).
- Shigematsu, N., Y. Okuhara, T. Shiomi, F. Tomita, and H. Hara. 2004. Effect of difructose anhydride III on calcium absorption in humans. *Biosci. Biotechnol. Biochem.* 68:1011–1016.
- Suzuki, T., and H. Hara. 2004. Various nondigestible saccharide open a paracellular calcium transport pathway with the induction of intracellular calcium signaling in human intestinal Caco-2 cells. *J. Nutr.* 134:1935–1941.
- Suzuki, T., and H. Hara. 2006. Diffructose anhydride III and sodium caprate activate paracellular transport via different intracellular events in Caco-2 cells. *Life Sci.* 79:401–410.
- Suzuki, T., H. Hara, T. Kasai, and F. Tomita. 1998. Effect of difructose anhydride III on calcium absorption in small and large intestine of rats. *Biosci. Biotechnol. Biochem.* 62:837–841.
- Udén, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cesium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31:625–632.
- Yamagishi, N., A. Yukawa, N. Ishiguro, S. Soeta, I. H. Lee, K. Obishi, and H. Yamada. 2002. Expression of calbindin-D9k messenger ribonucleic acid in the gastrointestinal tract of dairy cattle. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 49:461–465.
- Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2001. Barley processing, forage:concentrate, and forage length effects on chewing and digesta passage in lactating cows. *J. Dairy Sci.* 84:2709–2720.