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Comparison of Ruminal and Rectal Sampling Sites for Estimating Digesta Passage Kinetics in Rumen of Holstein Cows Fed Corn Stalk-Based Diets

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Abstract: Three ruminally cannulated Holstein cows in late lactation were used in a complete randomized experiment to compare runimal and rectal sampling sites for estimating digesta passage kinetics in the rumen of lactating Holstein cows. The experimental diets were consisted of chopped corn stalk and concentrate, for which the ratio of forage to concentrate was 55:45. Chromium-mordanted hay (Cr-NDF) was used as particulate marker and Cobalt-Ethylenediamine Tetraacetic Acid (Co-EDTA) as a liquid-phase marker and they were administered by one-time infusion through rumen cannulae to determine passage rates of the particulate (k_p) and liquid (k_l) phases from the rumen and rectum, respectively. Both rumen contents and faecal samples were collected to analyze Cr and Co concentration. Then, the fractional digesta passage rates were calculated by linear regression of natural log transformed Cr and Co concentrations in ruminal content or faeces. The results were showed as follows: fractional passage rates of solid and liquid digesta measured directly in the rumen were 0.0411/h and 0.112/h whereas they were 0.0387/h and 0.0573/h when calculated from the faecal Cr and Co concentration curves. The correlation equation between the fractional passage rates of liquid digesta from the rumen and rectum is $y(r) = 2.0141x(f) - 0.00399$, $R^2 = 0.9966$, $p = 0.037$ [y(r): Passage rate of liquid digesta measured directly in the rumen, x(f): Passage rate of liquid digesta calculated from the faecal Co concentration curve]. The correlation equation between the fractional passage rates of solid digesta from the rumen and rectum is $y(r) = 0.3535\,x(f) + 0.02743$, $R^2 = 0.8326$, $p = 0.27$ [y(r): Passage rate of solid digesta measured directly in the rumen, x(f): Passage rate of solid digesta calculated from the faecal Cr concentration curve]. Results suggest that measuring marker concentration curves in rectum always results in a considerable underestimation of the digesta passage rates from the rumen, however, the digesta passage rates from the rumen can be predicted by faecal marker concentration curves derived from the rectal sampling to some extent especially for liquid passage rate of digesta from the rumen in dairy cows.

Key words: Holstein cows, rumen, passage kinetics of digesta, sampling sites, China

INTRODUCTION

Digesta flow can be considered in terms of velocity (distance per time), flow rate or rate of passage (Warner, 1981). Velocity is applicable only to tubular segments of the Gastro Intestinal (GI) tract where it provides a measure of gut motility whereas rate of passage refers to the volume of mass of digesta passing a point in the GI tract per unit of time and its measurement in association with particular analyses allows estimates to be made of the extent digestion, absorption and/or secretion occurring in defined segments of the tract (Faichney, 1993).

Knowledge of the fractional outflow rates of rumen digesta constituents is required for the quantitative prediction of rumen function. Determination of digesta flow is also required for the estimation of microbial protein supply of ruminants. The rate of removal of digesta from the rumen affects the extent of ruminal protein degradation (Orskov and McDonald, 1979), cell wall digestion (Allen and Mertens, 1988) and the efficiency of microbial protein synthesis (Harrison and McAllan, 1980). Stained particles (Blaxter *et al*., 1956) and various external markers (Owens and Hanson, 1992) have been used to determine the rate of removal of particles from the rumen. Measurement of digesta flow requires some degree of surgical preparation of the animal and some indigestible markers. There is a wide variety of markers that can be used which have to be selected as far as possible

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according the criteria of an ideal marker. Inorganic markers such as Cr-mordanted NDF (Cr-NDF) and Co-EDTA are commonly used for measurement of solid and liquid passage rates, respectively through the gastrointestinal tract of ruminants. For estimating digesta passage kinetics using ruminal and faecal sampling allows to allocate the compartmental flow to different sites of the digestive tract and to evaluate the accuracy of using faecal concentration decline of markers to calculate passage rate of digesta from the rumen in lactating dairy cows. Taking animal welfare into account, it's better for researcher to using rectal sampling to study digesta passage kinetics in rumen then experimental animals can avoid of being suffered from surgery.

There are a lot of relative studying reports for bulls, steers and sheep. However, there are few data available for these parameters of dairy cattle especially those fed corn stalk as a main source of roughage in developing countries. Therefore, the objective of the present study was to determine the digesta passage rates from the rumen and rectum in Holstein cows fed diets based on poor roughage and to complement data of parameters required for quantitative prediction of rumen function and try to evaluate the method of using rectal grab sampling to estimate digesta passage rates from the rumen in lactating Holstein cows.

MATERIALS AND METHODS

Animals and diets: Three healthy lactating Holstein cows (milk production 14±2.5 kg day^{-1} body weight 590±6 kg, days in milk 210±5 day) installed permanent rumen cannulaes were used in the study. A complete diet consisted of chopped corn stalk and concentrate with forage: concentrate ratio of 55:45 (based on dry matter) was fed to the cows (Table 1). The concentrate mix was based on cracked corn grains, soybean meal, linseed meal, cottonseed cake and wheat bran and was completely mixed with the corn stalk before feeding. *Ad libitum* intake of this diet was determined in a 7 days preliminary period and the amount of feed Dry Matter Intake (DMI) was determined as about 15 kg day^{-1}. The nutritional level of trial diet was formulated according to the criteria described in NRC, 2001 Nutrient Requirements of Dairy Cattle. Holstein cows were fed diets at 1.2 times of maintenance level throughout the trial.

Table 1: Chemical composition and ingredient of the experimental diet (based on DM)

Ingredient	Percentage	Chemical composition	Percentage
Corn meal	27.36	DM	87.20
Soybean meal	4.56	NE_L[1] (MJ kg^{-1})	5.92
linseed meal	3.26	CP	10.65
Cottonseed cake	5.40	Ca	0.62
Wheat bran	1.69	P	0.37
$NaHCO_3$	0.52	NDF	45.35
$CaHPO_4$	0.85	ADF	27.83
NaCl	0.52	ADL	6.21
Edible powder	0.39	AIA	1.37
Premix	0.45		
Corn stalk	55.00		
Total	100.00		

NE_L[1]: Net Energy requirement for lactation (NRC in 2001 Nutrient Requirements of Dairy Cattle)

Housing and management: Experimental Holsteins were all placed in individual tie stalls in a metabolic lab and fed twice daily at 07:00 am and 07:00 pm with 6.75 kg concentrate and 8.25 kg corn straws every day. They were milked twice a day before feeding. Fresh water and mineral blocks were freely available through the experiment.

Experimental procedure: The experiment consisted of a measurement period of 1 week, preceded by diet adaptation period of 2 weeks. At the end of the diet adaptation period, Cr-NDF and Co-EDTA prepared by the procedure of Uden *et al.* (1980) were used as markers and administered to each cow through rumen cannulae by 1 time infusion. After administration, ruminal digesta and faecal samples were collected at different time points with 3 repeats to determine the liquid and solid digesta passage rates from the rumen and rectum, respectively.

Infusion and recovery of markers: On the 1st day of the experimental period and just before feeding, 100 g Cr-NDF and 50 g Co-EDTA were administered to each animal through rumen cannulae. Following administration of markers, rumen contents were sampled via the cannula at 3, 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 48, 54, 60, 72, 84, 96 and 120 h post-dosing. Rumen contents (solid digesta and rumen fluid) were weighed, thoroughly mixed and at least fifteen small samples were taken during mixing by hand to obtain representative samples. Rumen contents were returned immediately after sampling. A 10 g kg^{-1} sample of the total weight was dried at 65°C for 48 h, ground through a 2 mm screen and analysed for DM, ash and Cr concentration. Rumen evacuations were performed according to the procedure described by Robinson *et al.* (1987).

Ruminal fluid was obtained by straining rumen contents through four layers of cheese cloth. Duplicate subsamples (4 mL) were centrifuged (10,000×g; 15 min; 4°C) and supernatants prepared for Co concentration analysis.

Rectal grab samples were obtained at 32, 36, 48, 54, 60, 72, 84, 96 and 120 h after administration of markers. Individual faecal samples were dried at 65°C for 48 h and ground through a 2 mm screen. Dried faecal subsamples were composited by DM. Duplicate 2 g subsamples of faeces were frozen for determination of Cr and Co concentrations as described by Mir *et al.* (1991).

Calculations and statistical analyses: Liquid or solid passage rates were estimated as the slope of the linear regression between the natural log of the decreasing concentration of Co or Cr on time of sampling following marker administration as shown in the following equations. Assuming that rates of particle passage follow first-order kinetics, the fractional liquid passage rates were calculated from the logarithmic decline in Co concentration in the rumen fluid (k_l-Co-rumen) as well as from the descending part of the faecal concentration curve of Co (k_l-Co-faeces). The descending parts of the ruminal and faecal concentration curves of Cr were used, under the same assumptions, to calculate the fractional solid passage rate from the rumen (k_p-Cr-rumen) and from the rectum (k_p-Cr-faeces), respectively. The passage rates from the rumen and rectum were calculated by using the following equation. The relationship between passage rate from the rumen and passage rate from the rectum was analyzed by SAS (1985) using the regression procedures. Standard Error of Mean (SEM) and coefficient of determination (R^2) were used to evaluate the goodness of fit for using passage rate from the rectum to predict passage rate from the rumen:

$$Ct = C_0 \times e^{-kt} \quad (1)$$

Where:

- Ct = Concentration of Co or Cr at different sampling time
- C_0 = Concentration of Co or Cr at zero time point
- k = Liquid or solid passage rate
- t = Sampling time point

RESULTS

Liquid passage rates from the rumen: The curvilinear equations of exponential decline in ruminal and faecal Co concentration are given in Table 2. For the marker of Co-EDTA log plots a linear relationship was found both in the rumen and rectum (R^2(r): 0.9053, 0.9474, 0.9648; R^2(f): 0.9234, 0.9557, 0.9566). Fractional passage rates from the rumen of liquid digesta, derived from the descending parts of the faecal Co concentration curves as well as measured directly in the rumen are given in Table 3. The passage rate of the liquid phase was higher when measured directly in the rumen than when calculated from the faecal Co concentration curves. However, there was a significant correlation between k_l-Co-rumen and k_l-Co-faeces and the correlation equation is $y(r) = 2.0141x(f)-0.00399$ ($R^2 = 0.9966$, $p = 0.037$) (y(r): k_l-Co-rumen, x(f): k_l-Co-faeces). The results suggest that the passage rate from the rumen of liquid digesta can be derived from the method of rectal grab sampling by correcting it with some constant.

Table 2: The curvilinear equation of Co concentration with sampling time points at the rumen and rectum

Groups	1	2	3
Rumen	$y = 31.388e^{-0.1253x}$	$y = 23.972e^{-0.1073x}$	$y = 28.837e^{-0.1018x}$
	$R^2 = 0.9053$	$R^2 = 0.9474$	$R^2 = 0.9648$
Rectum	$y_1 = 159.219e^{-0.06616x}$	$y_2 = 128.375e^{-0.05487x}$	$y_3 = 149.035e^{-0.05544x}$
	$R^2 = 0.9234$	$R^2 = 0.9557$	$R^2 = 0.9566$

Table 3: Comparison of ruminal and rectal sampling sites used for estimating kinetics of liquid phase passage

	Liquid passage rate from the rumen and rectum		
Groups	Rumen	Rectum	Regression equation
1	0.1253	0.06426	$y(r) = 2.0141x(f)-0.00399$
2	0.1073	0.05487	$R^2 = 0.9966$
3	0.1018	0.05284	$p^2 = 0.037$
Mean	0.1115	0.05732	
SEM[1]	0.0041	0.00203	

[1]SEM = Standard Error of Mean; [2]p-value for linear response

Table 4: The curvilinear equation of Cr concentration with sampling time points at rumen and rectum

Groups	1	2	3
Rumen	$y_1 = 716.2e^{-0.0407x}$	$y_2 = 603.9e^{-0.0405x}$	$y_3 = 1100.3e^{-0.0421x}$
	$R^2 = 0.9744$	$R^2 = 0.9515$	$R^2 = 0.9709$
Rectum	$y_1 = 3478.6e^{-0.0387x}$	$y_2 = 3293.8e^{-0.0364x}$	$y_3 = 5714.3e^{-0.0409x}$
	$R^2 = 0.9757$	$R^2 = 0.9758$	$R^2 = 0.9844$

Table 5: Comparison of ruminal and rectal sampling sites used for estimating kinetics of solid digesta passage

	Solid passage rate from the rumen and rectum		
Groups	Rumen	Rectum	Regression equation
1	0.040700	0.03870	$y(r) = 0.3535x(f)+0.02743$
2	0.040500	0.03640	$R^2 = 0.8326$
3	0.042100	0.04090	$p^2 = 0.27$
Mean	0.041100	0.03870	
SEM[1]	0.000291	0.00075	

[1]SEM = Standard Error of Mean; [2]p-value for linear response

Solid passage rates from the rumen: The curvilinear equations of exponential decline in ruminal and faecal Cr concentration are given in Table 4. For the marker of Cr-NDF log plots a linear relationship was found both in the rumen and rectum (R^2(r): 0.9744, 0.9515, 0.9709; R^2(f): 0.9757, 0.9758, 0.9844). Fractional passage rates from the rumen of solid digesta, derived from the descending parts of the faecal Cr concentration curves as well as measured directly in the rumen are given in Table 5. The passage rate of the solid phase was higher when measured directly in the rumen than when calculated from the faecal Cr concentration curves. However, a good correlation between k_p-Cr-rumen and k_p-Cr-faeces was found but with no significance ($y(r) = 0.3535x(f)+0.02743$, $R^2 = 0.8326$, $p = 0.27$ (y(r): k_p-Cr-rumen, x(f): k_p-Cr-faeces)). The results suggest that it can not reach statistical significance when using faecal Cr concentration curves to predict the solid passage rate from rumen.

DISCUSSION

Liquid passage kinetics: For many studies, comparisons of sampling sites for measuring liquid passage rate have been inconsistent. Bosch *et al.* (1988, 1992) reported a 25-30% lower passage rate of liquid phase when derived from the faeces than when measured directly in the rumen. Cruickshank *et al.* (1989) reported a similar passage rate from ruminal and abomasal sampling but faecal sampling resulted in a slower passage rate than abomasal or duodenal sampling. Their simulation studies showed that the magnitude of the differences between sampling sites depends on the ratio of passage rate from the caecum and rumen. Gasa *et al.* (1991) found a lower Co outflow rate when measured in the duodenum compared with that measured directly in the rumen fluid. Bosch and Bruining (1995) reported that fractional liquid passage rates measured directly in the rumen was 45-50% higher than values calculated from the faecal Co concentration curves in cows. In the present study, the same observation was made that liquid passage rate from faecal sampling was around 48% lower than the value measured directly in the rumen of dairy cows which was similar to the result reported by Bosch and Bruining (1995). In addition, researchers also found that the k_l-Co-rectum was significantly correlated with k_l-Co-rectum in this experiment which indicated that researchers could use rectal grab sampling to determine liquid passage rate from the rumen by adding some constant.

Solid passage kinetics: The probability of feed particles escaping from the rumen is inversely related to particle size (Poppi *et al.*, 1980) but passage rate from the rumen of the smallest particles is still lower than that of the fluid (Faichney, 1986; Bruining and Bosch, 1992). In the present study, researchers found that liquid passage rate from the rumen was much higher than solid passage rate from the rumen (k_l-Co-rumen (mean): 0.112/h, k_p-Cr-rumen (mean): 0.0411/h). The results confirmed the conclusion of Faichney (1986) and Bruining and Bosch (1992).

Several methods are available to estimate the passage of digesta particles through ruminant gut. The passage rate of digesta particles can be estimated by a model with two age independent compartments and a time delay reported by Grovum and Williams (1973a, b). Pond *et al.* (1988) described the two compartmental models with gamma age dependency in the first compartment using non-linear procedures. However, in most earlier studies, the fractional passage rate of particles from the rumen was calculated from the faecal concentration curve of Cr-NDF (Aitchison *et al.*, 1986; Tamminga *et al.*, 1989). However, Bosch and Bruining (1995) reported a 25% lower passage rate of Cr-NDF when derived from the faeces than when measured directly in the rumen of cows. In this experiment, a delay similar to that of the soluble marker (Co-EDTA) was also found for the particulate marker (Cr-NDF) when measured in the faeces (k_p-Cr-faeces) compared with that measured directly in the rumen (k_p-Cr-rumen). However, in this case the difference was only 6%.

Comparison of sampling sites: For estimating digesta passage kinetics, using ruminal and faecal sampling when markers were given with the diet and determining ruminal digesta passage kinetics separately by introducing markers into the rumen allows to allocate the compartmental flow to different sites of the digestive tract and to evaluate the accuracy of using faecal concentration decline of markers to calculate passage rate of digesta from the rumen in lactating dairy cows. In the previous literature results reported about the effect of sampling sites on determining passage kinetics of digesta were different between pre-and post-duodenal digestive tract in ruminants.

As for rumen, an important pre-duodenal digestive organ in ruminants, it was not consistent with the effect of sampling sites on estimating passage kinetic parameters of ruminal digesta. Gasa *et al.* (1991) found a lower Co outflow rate when measured in the duodenum compared with that measured directly in the rumen fluid. Huhtanen and Kukkonen (1995) reported that liquid passage rate was higher ($p<0.001$) when calculated from the exponential decline in ruminal (mean 0.0770/h) than in duodenal (0.0665/h) or faecal (0.0621/h) Co concentration. Robinson *et al.* (1987) concluded that even faecal concentration curves of Cr-NDF must overestimate rumen feed particle passage rate as a fraction of total rumen contents because they sometimes even result in a higher passage rate than total rumen clearance rate. However, Bosch and Bruining (1995) reported that both liquid and solid passage rate from rumen were significantly higher when measured directly in the rumen than when calculated from the faecal concentration curves.

However, for post-duodenal gut, a same result was reported in most experiments that there was no significant difference between passage rates of digesta derived from duodenal and rectal sampling. Huhtanen and Kukkonen (1995) concluded that sampling site (duodenum vs. rectum) had no effect on both liquid and solid passage rate when non-linear models were used to estimate the parameters. Gasa *et al.* (1991) measured Cr concentration curves in the duodenum and in the faeces and found no differences between the two calculated passage rates in cows.

In the present study, researchers found that sampling sites (rumen vs. rectum) did have effect on estimating digesta passage kinetics in rumen and both liquid and solid passage rates of digesta measured directly in the rumen were generally higher than those calculated from the exponential decline in faecal marker concentration (k_l-Co-rumen (mean): 0.112/h, k_l-Co-rectum (mean): 0.0573/h; k_p-Cr-rumen (mean): 0.0411/h, k_p-Cr-rectum (mean): 0.0387/h). The results supported the view of Bosch and Bruining (1995) that measuring marker concentration curves not directly in the rumen but further down the gut, results in a considerable underestimation of the marker outflow rate from the rumen. However, researchers also observed a significant correlation between k_l-Co-rumen and k_l-Co-rectum (R^2 = 0.9966, p = 0.037) and a good but not significant correlation between k_p-Cr-rumen and k_p-Cr-rectum (R^2 = 0.8326, p = 0.27). It seems that researchers can use rectal grab sampling to estimate passage rates of liquid and solid phrases of digesta in the rumen of high producing cows by applying the regression equations researchers presented in this study, especially for liquid passage rate of digesta from the rumen in dairy cows.

CONCLUSION

Passage rates of digesta from rumen calculated from the exponential decline of faecal concentration of marker will underestimate the results directly measured in the rumen. However, the digesta passage rates from the rumen can be predicted by faecal marker concentration curves derived from the rectal sampling to some extent, especially for liquid passage rate of digesta from the rumen in dairy cows and there is a significant correlation between the passage rate of liquid from rumen and passage rate from rectum of digesta in lactating dairy cows.

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