

# Increased Fecal Ethanol and Methanol Concentration in Dairy Heifers after Grazing

## Short Communication

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## ABSTRACT

To clarify fermentative alterations in the large intestine (hindgut) during grazing, fecal alcohol and volatile fatty acids (VFAs) concentrations were evaluated in 30 dairy heifers. The heifers were kept in pasture growing mixed grass herbage from spring (mid May) until October, and their rectal feces were collected periodically. Concentrations of ethanol, methanol and isopropanol were increased in runny feces at early stage of grazing, but gradually decreased thereafter, despite the fact that ethanol remained at a high level until early October. Fecal VFA concentration was barely influenced at early grazing stage; however, it showed marked decrease thereafter. Hindgut exposure of increased nutrients which escaped upper tract digestion might promote active alcoholic fermentation in the colon of the heifers, particularly during early grazing period when runny defecation happens frequently.

**KEY WORDS** cattle feces, fecal alcohol, grazing, hindgut fermentation.

## INTRODUCTION

Ingested feeds by cattle, particularly fibrous herbage, are exposed to microbial degradation not only in the rumen but also in the large intestine (hindgut) which has a higher fermentative capacity for fibrous feeds (Hoover, 1978). Many dairy heifers in Hokkaido, northern island of Japan, are reared on grazing pastures during spring to autumn. In grazing livestock who consumes a large quantity of herbage, a large quantity of fibrous nutrients which escape upper tract digestion is exposed to hindgut microbes for fermentation. Similar to the rumen, hindgut digestion produces volatile fatty acids (VFAs) including acetate, propionate and butyrate, together with their intermediates like alcohols and lactate in the fermentation cascades. Compared with VFAs, the concentration of these intermediates in the gut has wide fluctuations generally (Macfarlane and Macfarlane, 2003). Interestingly, ethanol in feces is considerably higher than that in the rumen of lactating dairy cows, despite slight difference in their VFA concentration between feces and ru-

menal fluid (Sato and Shioyama, 2010). Thus, the hindgut fermentation of fibrous nutrients is of particular interest concerning cattle fed mainly on herbage or grazing. However, little information is available on fermentative intermediates in the hindgut of grazing cattle, in spite of vast studies on production and metabolism of VFAs. This study aimed to elucidate the fecal concentration of alcohols (methanol and ethanol) together with VFAs in relation to colonic fermentation of heifers grazed during spring through autumn seasons.

## MATERIALS AND METHODS

### Animals and grazing

A total of 168 Holstein heifers were brought from 27 local dairy farms into a grazing pasture of Chitose city in central Hokkaido, the northern island of Japan, in mid May, 2010, approximately one week after the annual cherry blossoming. The heifers were aged 7 to 12 months on arrival, and

they were kept in the pasture a whole day until mid October. They had been previously dehorned and vaccinated on their respective farms. The pasture was nearly flat and the soil included volcanic ash. A mixture of mainly orchard grass, timothy, meadow fescue with a few clovers constituted the herbage, but their proportions were not examined. The pasture was used for both grazing and conserved forage production, although the grazing area for the heifers was 34 hectares (ha) throughout the season. For rotational grazing, the grazing area was divided into 4 sub-areas (3, 10, 10 and 11 ha) sharing 0.4 ha mutual paddock with watering trough. Rotation interval was varied by herbage residue in each area. No feed was supplemented, except free access to salt block of trace minerals (selenium, zinc, copper, cobalt and others). Animals at puberty were artificially inseminated in the pasture, and those fertilized were taken away from the pasture. Body weight was measured by electric load cell at week (wk) 1 (May) after the grazing and again at wk 20 (October).

### Sampling of feces and herbage

Among 168 heifers in grazing, 30 individuals were selected for fecal analyses throughout the season. Fecal samples were collected forenoon by rectal grabbing at wk 1, 5, 9, 14 and 20 in grazing (27 heifers at wk 20). Herbage samples (2-3 kg) were also collected on the day of fecal collection for chemical analyses. In the sampling, the herbage was cut at approximately 5 to 10 cm height in several swards (5-10) of the pasture on which the heifers grazed. Also before grazing, fecal samples were taken from 25 heifers housed in four dairy farms in which grass hay or haylage were used as main feeds, despite no qualitative examination of the feeds in the farms.

### Analyses

Fecal samples were extracted with 4 parts of water and centrifuged at 2000 g for 10 min. The resulting supernatants were frozen at  $-30^{\circ}\text{C}$  until analysis. Using these fecal extracts, methanol, ethanol and isopropanol were determined by gas chromatography (GC-2014; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and using 2-pentanol as an internal standard. Gas chromatography was also employed for analyzing VFAs using crotonic acid as an internal standard. Dry matter (DM) of the fresh herbage was evaluated by drying in a forced air oven at  $110^{\circ}\text{C}$  for 6 h, and the resulting dried herbage was ground by a blending mixer for chemical evaluation. Neutral detergent fiber (NDF; total fiber), crude protein (CP) and ash of the above herbage samples were analyzed by a detergent method using sodium laurylsulfate (Van Soest *et al.* 1991), indophenol colorimetry (Sato and Nakajima, 2005) after the Kjeldahl digestion, and ashing at  $600^{\circ}\text{C}$  for 5 h in a muffle furnace, respectively.

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### Statistics

Owing to non-Gaussian distribution of some data and different sample size, statistical differences among the grazing stages were examined by using the Kruskal-Wallis test with the Scheffe rank test, and correlations between fecal concentrations of fermentative products were evaluated on the aggregate data using Spearman's correlation coefficient rank test (Freund, 1988).

## RESULTS AND DISCUSSION

The pasture experienced frequent rain in July and August, and encountered extremely hot conditions in August and early September (Table 1). Some heifers might have suffered severe heat stress in the summer. Dry matter, CP, NDF and ash contents of the herbage taken in the pasture are given in Table 2. Herbage DM was slightly higher at wk 1(May); though the herbage was lively at vegetable stage as showing lower ash content. At wk 5 (June), the herbage which included largely regrowth ones had the lowest CP and highest NDF contents.

**Table 1** Ambient temperature and rainfall in the pasture

Season	Temperature			Rainfall (mm)
	Mean( $^{\circ}\text{C}$ )	Max( $^{\circ}\text{C}$ )	Days>>25 $^{\circ}\text{C}$ (Max)	
May	10.3	20.9	0	134
Jun	16.2	31.4	5	54
Jul	20.2	29.4	10	140
Aug	22.7	30.6	25	208
Sep	18.2	30.5	10	112
Oct	10.6	21.2	0	94

All the heifers excreted diarrheic feces of nearly code 3 (runny; Larson *et al.* 1977) after grazing, particularly at wk 5 and 9. This fecal status during spring pastures has long been well known generally (Miller, 1979) as "normal or physiological diarrhea". Actually, an averaged body weight gain throughout the grazing period (1 to 20 wk) was 0.99 (SD 0.29) kg daily in the heifers which were used for the fecal sampling, despite a large variation in growth performance among the individuals.

Fecal alcohols and VFAs concentrations are shown in Table 3. Feces taken before grazing had  $1.12\text{ mmol L}^{-1}$  of ethanol and  $0.46\text{ mmol L}^{-1}$  of methanol in average (on a fresh basis); however, trace amounts of isopropanol ( $0.01$  to  $0.03\text{ mmol L}^{-1}$ ) were also present. At wk 5 (June) to 9 (July), methanol and ethanol concentrations were increased markedly accompanying elevation was also seen in isopropanol concentration. Fecal concentration of ethanol had a negative correlation with VFAs ( $r=-0.49$ ,  $P<0.01$ ), and a

**Table 2** Chemical composition of herbage samples in grazing pasture

Grazing stage		DM <sup>1</sup>	CP <sup>2</sup>	NDF <sup>3</sup>	Ash
(Week on pasture)	(Season)	(%)		(% DM)	
1	Late May	24.3	16.8	57.4	5.9
5	Late Jun	20.8	12.9	65.4	6.3
9	Late Jul	18.9	20.5	59.5	9.7
14	Middle Aug	22.1	18.2	58.2	9.8
20	Early Oct	21.9	18.5	52.1	9.8

<sup>1</sup>Dry matter; <sup>2</sup>Crude protein; <sup>3</sup>Neutral detergent fiber.

positive one with isopropanol concentration ( $r=0.50$ ,  $P<0.01$ ). Ethanol is an intermediate in the global cascade of VFA production in gut fermentation (Macfarlane and Macfarlane, 2003); this metabolic pathway might explain the negative correlation between ethanol and VFA concentrations.

Higher fecal ethanol in the lactating cows might probably reflect hindgut exposure of cereal carbohydrates which could escape ruminal digestion.

Methanol and isopropanol concentrations showed a gradual decrease thereafter, despite maintaining of higher concentration in ethanol throughout the grazing period. Little attention has been paid to gut methanol in domestic animals and also in humans until now. Methanol is more harmful than ethanol to the host animals (Ekins *et al.* 1985; Majchrowicz and Mendelson, 1971); therefore colonic microbes might shift in favor of alleviating production of methanol and also isopropanol in advancing of grazing. Considering the easy absorption of short-chain alcohols from the gut mucosa, it has been proposed that one of the physiological significance of hepatic alcohol dehydrogena-

**Table 3** Fecal concentration of alcohols and volatile fatty acids (VFAs) in dairy heifers

Grazing stage		Methanol	Ethanol	Isopropanol	VFAs	Acetate	Propionate	<i>n</i> -Butyrate
		(mmol L <sup>-1</sup> )				(Molar % of VFAs)		
Before (n=25)	Mean	0.46 <sup>a</sup>	1.12 <sup>a</sup>	0.02 <sup>ab</sup>	72 <sup>c</sup>	67.0 <sup>a</sup>	22.0 <sup>b</sup>	7.2 <sup>d</sup>
	SD <sup>1</sup>	0.21	0.51	0.01	18	3.1	2.0	1.3
1 week (n=30)	Mean	0.53 <sup>a</sup>	1.21 <sup>a</sup>	0.01 <sup>a</sup>	87 <sup>d</sup>	66.7 <sup>a</sup>	23.9 <sup>b</sup>	4.8 <sup>bc</sup>
	SD	0.36	0.58	0.01	19	3.4	2.8	1.1
5 week (n=30)	Mean	1.37 <sup>cd</sup>	3.08 <sup>b</sup>	0.05 <sup>dc</sup>	62 <sup>bc</sup>	74.8 <sup>cd</sup>	16.8 <sup>a</sup>	4.3 <sup>ab</sup>
	SD	0.63	1.18	0.03	13	2.1	1.1	0.8
9 week (n=30)	Mean	2.03 <sup>d</sup>	2.34 <sup>b</sup>	0.04 <sup>d</sup>	56 <sup>bc</sup>	77.0 <sup>d</sup>	15.5 <sup>a</sup>	3.6 <sup>a</sup>
	SD	0.67	0.43	0.02	12	1.5	0.6	0.9
14 week (n=30)	Mean	0.99 <sup>b c</sup>	3.27 <sup>b</sup>	0.02 <sup>abc</sup>	37 <sup>a</sup>	74.9 <sup>cd</sup>	15.2 <sup>a</sup>	4.7 <sup>bc</sup>
	SD	0.42	1.18	0	9	2.0	1.4	1.6
20 week (n=27)	Mean	0.67 <sup>ab</sup>	2.51 <sup>b</sup>	0.02 <sup>abc</sup>	38 <sup>a</sup>	73.2 <sup>bc</sup>	17.2 <sup>a</sup>	5.6 <sup>cd</sup>
	SD	0.21	0.71	0.01	12	2.0	1.7	0.9

<sup>1</sup>Standard deviation.

<sup>a</sup>The means in the same column that have at least one common letter, do not have significant difference ( $P>0.05$ ).

Increased alcohols by grazing attracted high interest. The heifers were fed forage like hay or haylage before grazing, despite no evaluation of the forage qualities. The increased production of alcohols cannot be explained well; however, a fermentative change in the hindgut must be brought by grazing factors including herbage intake and resulting digestion. Among the many factors concerning hindgut fermentation, we noticed two possibilities. The first is increased hindgut inflow of nutrients, particularly fibrous carbohydrate. Diarrheic status in early grazing would enable the escape of nutrients from the ruminal digestion and bring their fermentation in the hindgut in which large amounts of intermediates such as alcohols might be produced. Similarly, fecal ethanol is much higher in winter wild deer that depend greatly on fibrous bark for their nutrients in snow-covered forest (Sato, 2010). The next is a growth stage of spring herbage which includes higher amount of fermentative polysaccharide (Osborn, 1989). These food habitats might bring accelerated alcoholic production in the hindgut. However, the present ethanol concentration even in grazing was approximately half the range of lactating dairy cows (4.1-5.6 mmol L<sup>-1</sup>) which were fed diets including concentrate (Sato and Shiogama, 2010).

se is to detoxify the alcohols which are produced by gut microbes (Krebs and Perkins, 1970).

Fecal VFA concentrations before grazing (72 mmol L<sup>-1</sup>) were lower than those in housed lactating dairy cows (112 mmol L<sup>-1</sup>; Sato and Shiogama, 2010), but higher than those of grazing dairy cows in lactation (50 mmol L<sup>-1</sup>; Sato and Nakajima, 2005). The VFA concentration increased transiently at wk 1, but it decreased gradually toward summer. The decreased VFAs concentration in summer season (wk 14 and 20; August and early October) is considered to be brought about not only by heat stress (Table 1) accompanying probable reduction of herbage intake but also by decreasing quality of the herbage. In addition to higher acetate and lower propionate proportions in VFAs before grazing, these trends were more dramatic at wk 5 and after grazing. Furthermore, the present value of acetate proportion after wk 5 (>73%) was extremely higher than that in feces of lactating dairy cows (62-68%) given mixed diet of forage and concentrate (Sato and Shiogama, 2010). These trends in molar proportions of fecal VFAs by grazing are in agreement with those in ruminal VFAs proportions which were observed by feeding on higher forage or fibrous feeds (Ørskov and Ryle, 1990). Proportion of *n*-butyrate de-

creased promptly at wk 1 to 14 in grazing (Table 3). This cannot be explained well; however, diarrheic status by grazing would be associated with the decrease of *n*-butyrate. Knowledge on the physiological significance of *n*-butyrate for maintaining gut health and function is increasing widely (Topping and Clifton, 2001; Williams *et al.* 2001).

To conclude, this study demonstrated accelerated colonic production of ethanol and methanol by grazing, in spite of moderate changes in total VFA concentration. These profiles would indicate an increased exposure of nutrients to the hindgut. Fecal methanol decreased thereafter, despite maintaining higher ethanol until the grazing end. Higher acetate and lower propionate proportions in fecal VFAs were strengthened during grazing.

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