Effects of short-term fasting on ruminal pH and volatile fatty acids in cattle fed high-roughage versus high-concentrate diets

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ABSTRACT. We evaluated whether the dietary roughage-to-concentrate ratio affects ruminal pH and volatile fatty acids (VFAs) in response to a one-time morning fast. Four healthy rumen-cannulated Holstein steers 4–5 months old were used. Cattle were subjected to 2 weeks of adaptation (high-roughage or high-concentrate diet), and morning feed restriction was performed on the day after the adaptation period ended (Day 0). Thereafter, each diet was reintroduced on the evening of Day 0. Our results showed that the 1-hr mean ruminal pH from 0800 to 1900 on Day 0 was higher, and that from 1700 to 1900 on Day 1 was lower ($P<0.05$) than pH on 1 day before fasting (Day −1) in cattle fed both diets. On Day 0, total VFA levels decreased after morning fasting and were lower ($P<0.05$) than those on Day −1 irrespective of evening refeeding. Furthermore, blood non-esterified fatty acid and beta-hydroxybutyric acid levels on Day 0 increased and decreased, respectively, compared to Day −1 in cattle fed both diets. These results indicate that even a one-time feed restriction can disrupt ruminal fermentation, and the changes can persist to the next day after fasting.

KEY WORDS: cattle, ruminal pH, short-term fasting, subacute ruminal acidosis (SARA), volatile fatty acid (VFA)

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Short-term feed restriction, in which cattle are accidentally limited access to feed and water, is a frequent issue in conventional dairy and beef production systems, in particular during the weaning period [12] and transport [14]. Short-term feed restriction also occurs in several cattle metabolic diseases [13] or digestive disorders, such as subacute ruminal acidosis [22]. Furthermore, feed restriction affects ruminal fermentation, animal performance, and health [9]; therefore, the relationships among feed restriction period, feeding diets, ruminal parameters, and blood metabolites have been investigated. The severity of feed restriction depends on the amount of feed available [10, 11], the duration of the restriction, and the nutrient density of the diet [28]. The 2–7 days feed restriction induces a negative energy and protein imbalance and alters hepatic gluconeogenesis and mobilization of adipose tissue [5, 27]. Moreover, 5 days of feed restriction at 25% of ad libitum dry matter intake compromises reticulorumen absorptive and total tract barrier functions [29, 30].

Short-term fasting affects ruminal fermentation and blood components [1, 2, 16]. Fasting for 2 days reduces absorptive capacity of volatile fatty acid (VFA) and barrier function of the ruminal epithelium [29, 30] and alters levels of blood leptin, hormones, and metabolites, although these recover rapidly when feed is reintroduced [1, 2]. However, during veterinary treatment, short-term fasting (i.e., complete feed deprivation during one or two feeding times) is enforced in cattle before and after surgery to treat a displaced abomasum, ruminal tympany, or impaction, there are no reports on ruminal pH and VFA levels associated with short-term fasting in cattle. Further, it is unknown whether short-term fasting comprising a one-time feed restriction and/or diet differences before fasting affects ruminal fermentation. Therefore, we evaluated the effects of one-time feed fasting on ruminal fermentation and blood metabolites. The objective was to evaluate whether the dietary roughage-to-concentrate ratio affects ruminal pH and VFAs in response to a one-time feed restriction. We hypothesized that a single episode of fasting may have only minor negative effects on ruminal fermentation even in cattle fed a high-concentrate diet (HC-diet).
MATERIALS AND METHODS

Animals and experimental design

The experimental protocol was approved by the Iwate University Laboratory Animal Care and Use Committee (A201202; Morioka, Japan). Four healthy rumen-cannulated Holstein steers 4–5 months old with body weights of 120–140 kg were used in this study. Rumen cannulas were installed in all cattle via cannulation surgery 1 month after birth. For the first experiment, the cattle were fed calf starter or concentrate with forage until 2 weeks before the experiment and were housed individually in pens throughout the experimental period. The cattle were subjected to 2 weeks of adaptation using a high-roughage diet (HR-diet), then feed was withheld on the morning of Day 0. The HR-diet was reintroduced in the evening on Day 0, and the cattle continued to feed on the HR-diet on Day 1. Thereafter, the cattle were switched to an HC-diet for 2 weeks (second experiment). A morning feed restriction was performed again on Day 0; the HC-diet was reintroduced in the evening on Day 0 and continued on Day 1. Each episode of one-time fasting involved withholding feed during the morning feeding period, and the diets were reintroduced in the evening with half the amount of one day’s feed. The cattle were given 1.9–2.0 kg feed twice daily at 0800 and 1700 and allowed free access to fresh water. The forage-to-concentrate ratios in the HR- and HC-diets were 93:7 and 44:56, respectively (Table 1).

Measurement of ruminal pH

Ruminal pH was measured with a radio transmission system, as reported previously [25, 26]. The pH sensor (YCOW-S; DKK-TOA Yamagata, Shinjo, Japan) was calibrated at the start of each experiment with pH 4 and 7 buffer solutions and then placed in the ventral sac of the rumen through the rumen cannula. Ruminal pH was recorded continuously every 10 min throughout each experiment. We confirmed that the pH sensor was located in the ventral sac of the rumen via palpation throughout each experiment. Data from one day before (Day −1) to one day after fasting (Day 1) were analyzed.

Sampling and measurements

Ruminal fluid and peripheral blood were collected at 0800, 1100, 1400, 1700, and 2000 on Day −1 and on the day of fasting (Day 0). Ruminal fluid was sampled near where the pH sensor was located through the rumen cannula. The ruminal samples were immediately filtered through two layers of cheesecloth for analyses of VFA and ammonia-nitrogen (NH₃-N). For total and individual VFAs, 10 ml ruminal fluid was added to 2 ml 25% metaphosphoric acid in 3 N sulfuric acid before separation and quantification via gas chromatography (GC-2014; Shimazu, Kyoto, Japan) with a packed-glass column (Thermon-3000; 3%) with a Shimalite TPA 60–80 mesh support (Shinwa Chemical Industries, Kyoto, Japan). NH₃-N was determined via steam distillation with an automatic N analyzer (Tecator Kjeltec Auto 1035 Sample System; FOSS, Hillerød, Denmark).

Blood was sampled from jugular veins into 10 ml evacuated serum-separator tubes and 2 ml evacuated sodium fluoride tubes (Vacutainer; BD, Franklin Lakes, NJ, USA), and immediately centrifuged (1,500 × g, 15 min, 4°C) to separate the serum and plasma. Then the samples were stored at −80°C until analyzed. Glucose (GLU), total cholesterol (T-CHO), blood urea nitrogen, non-esterified fatty acid (NEFA), and β-hydroxybutyric acid (BHB) were measured using an automated analyzer with colorimetric assay (Accute; Toshiba, Tokyo, Japan).

Statistical analyses

One-way repeated measures analysis of variance followed by Dunnett’s multiple comparison test was used to determine whether there were significant differences in 1 hr mean pH between Days −1 (before fasting), 0 (during fasting), and 1 (after fasting). Student’s t test was performed to assess differences in circadian patterns at the same time points. All numerical data are expressed as means ± standard errors. \(P<0.05\) was taken to denote statistical significance.

RESULTS

Circadian changes in ruminal pH

On Day −1, the 1-hr mean pH in both diet groups decreased from the morning feeding (0800) and increased to 2 hr after the evening feeding (1900), thereafter, the values decreased and increased similarly until the next morning. In the HC-diet-fed cattle, the 1-hr mean pH dropped markedly during the first 2 hr following the morning feeding and decreased further until 2 hr after the evening feeding compared to the HR-diet-fed cattle (Fig. 1).

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### Table 1. Composition of the high roughage diet (HR-diet) and high concentrate diet (HC-diet) on a dry matter (DM) basis

| Item                  | HR-diet | HC-diet |
|-----------------------|---------|---------|
| Ingredient (%)        |         |         |
| Orchard and timothy hay | 92.7    | 43.8    |
| Corn flake            | 3.5     | 27.9    |
| Barley flake          | 3.5     | 27.9    |
| Salt                  | 0.15    | 0.15    |
| Vitamin/trace mineral | 0.08    | 0.08    |
| DM (%)                | 86.6    | 86.0    |
| DM basis (%)          |         |         |
| TDN                   | 62.8    | 75.1    |
| ADF                   | 37.9    | 20.2    |
| NDF                   | 64.1    | 37.7    |
| NFC                   | 12.5    | 42.4    |
| CP                    | 12.9    | 12.2    |
| Starch                | 7.0     | 37.0    |

TDN, total digestible nutrients; ADF, acid detergent fiber; NDF, neutral detergent fiber; NFC, non fibers carbohydrate; CP, crude protein.
On Day 0, the 1-hr mean ruminal pH in both HR-diet-fed (0800–2100) and HC-diet-fed (1000–2100) cattle were higher ($P<0.05$) than those on Day −1. However, the decrease in ruminal pH after the evening feeding was larger in cattle fed the HC-diet than the HR-diet, similar to the trend observed on Day −1 in both diet groups. Comparing the pH on Day 1 to Day −1, lower ($P<0.05$) values were observed after the evening feeding (1700–1900) in both diet groups. However, changes in the 1-hr mean pH throughout the day without 1700–1900 were similar in the HR-diet-fed cattle (Fig. 1, left), and the decrease after the morning feeding continued until 1800 in the HC-diet-fed cattle (Fig. 1, right).

**Total VFA, individual VFAs, and NH$_3$-N**

On Day −1, when compared to 0800, the total VFA concentrations increased ($P<0.05$) at 1100–1700 in the HC-diet-fed cattle, the proportions of acetic and butyric acid and the ratio of acetic acid to propionic acid decreased ($P<0.05$), and the proportion of propionic acid increased ($P<0.05$) temporarily after the morning feeding in both diet groups (Table 2). The NH$_3$-N concentrations decreased ($P<0.05$) in the HR-diet-fed cattle (1400 and 2000) and increased ($P<0.05$) in HC-diet-fed cattle (1100).

On Day 0, the total VFA concentrations decreased ($P<0.05$) at 1400–2000 in HR-diet-fed cattle and at 1100–2000 in HC-diet-fed cattle compared to 0800, the values were lower ($P<0.05$) than those at the same time points on Day −1. Compared to 0800, the proportion of acetic acid decreased ($P<0.05$) at 1100 and increased ($P<0.05$) at 1700 in the HR-diet-fed cattle, and increased ($P<0.05$) at 1400–1700 in the HC-diet-fed cattle, the values in HR-diet-fed cattle were higher ($P<0.05$) than those at the same time points on Day −1. Relative to the levels on Day −1, similar changes were observed in the proportion of propionic acid and ratio of acetic acid to propionic acid, whereas the proportion of butyric acid was stable and the value at 1100 in HR-diet-fed cattle was higher ($P<0.05$) than those on Day −1. Furthermore, the NH$_3$-N concentrations decreased ($P<0.05$) compared to 0800 at 1400–1700 in HR-diet-fed cattle, the values at 1100 in both diet groups were lower ($P<0.05$), and the value at 1400 in HC-diet-fed cattle was higher ($P<0.05$) than their respective values on Day −1 at the same time points.

**Blood metabolites**

On Day −1, compared to levels at 0800, GLU concentrations decreased ($P<0.05$) at 1400 in HR-diet-fed cattle, and T-CHO concentrations decreased ($P<0.05$) at 1100 and 2000 in HR-diet-fed cattle and at 1100 in HC-diet-fed cattle (Table 3). NEFA levels decreased ($P<0.05$) at 1100 in HR-diet-fed cattle and at 1100–1700 in HC-diet-fed cattle. BHB concentrations increased ($P<0.05$) at 1100 and 1400 in HR-diet-fed cattle and at 1100 and 1700 in HC-diet-fed cattle, but decreased ($P<0.05$) at 2000 in HR-diet-fed cattle.

On Day 0, the NEFA levels increased ($P<0.05$) at 2000 in HR-diet-fed cattle, and at 1700 and 2000 in HC-diet-fed cattle, BHB concentrations decreased ($P<0.05$) at 1400 in HC-diet-fed cattle. Furthermore, compared to the respective values at the same time points on Day −1, T-CHO concentrations were higher ($P<0.05$) at 1100 in HC-diet-fed cattle, NEFA levels were higher ($P<0.05$) at 1100 and 2000 in HR-diet-fed cattle and at 1700 and 2000 in HC-diet-fed cattle, but were lower ($P<0.05$) at 0800 in HC-diet-fed cattle. In addition, BHB concentrations were lower ($P<0.05$) at 1100 in HR-diet-fed cattle and at 1100 and 1700 in HC-diet-fed cattle, but were higher ($P<0.05$) at 0800 in HC-diet-fed cattle.

**DISCUSSION**

Ruminal pH is determined by the balance between the production of acid and its removal by absorption, neutralization, and
clearance from the rumen [4], and circadian changes in ruminal pH occur according to interactions among these processes [15, 17, 23]. In this study, circadian changes in ruminal pH on Day −1 were observed in cattle fed both HR- and HC-diets, with the minimum 1-hr mean pH lower in cattle fed the latter diet. In one study, the minimum pH decreased less in cattle restricted to 25% of their usual feed amount compared to those restricted to 75% [29]. Furthermore, pH decreased to a greater extent in cattle restricted to 75% of their usual feed amount compared to those restricted to 25% and 50% [29]. After fasting on Day 0, the 1-hr mean pH was maintained at higher levels than before fasting until the evening feeding time. Therefore, a one-time short-term fast may inhibit a decrease in ruminal pH, likely because VFA production is decreased by the feed restriction [24].

Circadian changes in pH between the morning and evening feeding time points were similar between Days 1 and −1, with pH decreasing significantly just after evening feeding at 1700–1900 in cattle fed both diets. These pH decreasing were more marked in HC-diet-fed cattle. However, the ruminal VFA on Day 1 was not investigated in the present study. The lower 1-hr mean pH at pre- and post-evening feeding on Day 1 may relate to the higher VFA concentration by fermentation of concentrate rich diet (HC-diet), because the negative correlation was recognized between the ruminal pH and VFA concentration in cattle [24]. Our findings indicate that the effects of one-time fasting on ruminal fermentation may still be present the next day after fasting. However, how

### Table 2. Total volatile fatty acid (VFA), individual VFA proportions, acetic acid to propionic acid (A/P) ratio, and NH$_3$-N concentration in cattle fed the high roughage (HR-diet) and high concentrate (HC-diet) diets

| Item                          | HR-diet       | HC-diet       |
|-------------------------------|---------------|---------------|
| Total VFA (mmol/dl) Day −1     | 8.68          | 9.77          |
| 1100                          | 11.60         | 12.30         |
| 1400                          | 9.75          | 13.90         |
| 1700                          | 9.65          | 11.60         |
| 2000                          | 9.18          | 9.28          |
| SEM                           | 0.61          | 0.82          |
| Acetic acid (%) Day −1         | 74.7          | 71.0          |
| 1100                          | 72.0(b)       | 67.1(a)       |
| 1400                          | 73.8          | 67.2(a)       |
| 1700                          | 73.9          | 69.4          |
| 2000                          | 74.4          | 70.2          |
| SEM                           | 0.5           | 1.3           |
| Propionic acid (%) Day −1      | 16.9          | 69.5          |
| 1100                          | 18.1(a)       | 72.8(a)       |
| 1400                          | 17.0          | 72.7(a)       |
| 1700                          | 17.2          | 72.1          |
| 2000                          | 17.2          | 72.1          |
| SEM                           | 0.3           | 1.8           |
| Butyric acid (%) Day −1        | 1.1           | 1.2           |
| 1100                          | 0.9           | 0.8           |
| 1400                          | 0.7(a)        | 0.8           |
| 1700                          | 0.8           | 0.9           |
| 2000                          | 0.9           | 0.9           |
| SEM                           | 0.1           | 0.1           |
| A/P ratio Day −1               | 4.45          | 3.96          |
| 1100                          | 3.99(a)       | 3.31          |
| 1400                          | 4.37          | 3.39          |
| 1700                          | 4.32          | 3.75          |
| 2000                          | 4.34          | 3.69          |
| SEM                           | 0.09          | 0.42          |
| NH$_3$-N (mg/dl) Day −1        | 9.74          | 4.73          |
| 1100                          | 12.20         | 7.62(a)       |
| 1400                          | 4.85(a)       | 2.93          |
| 1700                          | 8.28          | 3.59          |
| 2000                          | 7.45(a)       | 4.53          |
| SEM                           | 1.15          | 0.65          |
| a) Denotes significant difference (P<0.05) compared with 0800 in each day, b) denotes significant difference (P<0.05) between Day −1 and Day 0 at the same time point. 1) Time of day, 2) Day −1 and Day 0 denote observations during the 1 day before and fasting day in each diet.

### Table 3. Peripheral blood glucose (GLU), total cholesterol (T-CHO), non-esterified fatty acid (NEFA), and beta-hydroxybutyric acid (BHB) in cattle fed the high-roughage diet (HR-diet) and high-concentrate (HC-diet) diets

| Item                          | HR-diet       | HC-diet       |
|-------------------------------|---------------|---------------|
| GLU (mg/dl) Day −1             | 86.8          | 97.9          |
| 1100                          | 82.6          | 92.5          |
| 1400                          | 80.0(b)       | 88.7          |
| 1700                          | 79.8          | 91.8          |
| 2000                          | 81.5          | 87.9          |
| SEM                           | 3.2           | 88.6          |
| T-CHO (mg/dl) Day −1           | 84.6          | 64.7          |
| 1100                          | 84.6          | 69.6          |
| 1400                          | 83.5          | 63.1          |
| 1700                          | 84.4          | 62.6          |
| 2000                          | 84.0          | 64.9          |
| SEM                           | 2.1           | 8.8           |
| NEFA (µEq/l) Day −1            | 81.7          | 68.0          |
| 1100                          | 79.7          | 64.8(b)       |
| 1400                          | 83.3          | 66.9          |
| 1700                          | 83.8          | 67.1          |
| 2000                          | 84.5(b)       | 66.4          |
| SEM                           | 12.9          | 10.1          |
| BHB (µmol/l) Day −1            | 207.0         | 138.0         |
| 1100                          | 134.8(b)      | 84.5(a)       |
| 1400                          | 134.0         | 73.3(b)       |
| 1700                          | 228.0         | 82.8(b)       |
| 2000                          | 293.5         | 185.5         |
| SEM                           | 19.5          | 20.5          |
| a) Denotes significant difference (P<0.05) compared with 0800 in each day, b) denotes significant difference (P<0.05) between Day −1 and Day 0 at the same time point. 1) Time of day, 2) Day −1 and Day 0 denote observations during the 1 day before and fasting day in each diet.
much the pH decreases when the cattle resume feeding depends on the severity of feed restriction, and dramatic changes in pH and dietary intake have been observed when cattle start feeding again after fasting [29]. Therefore, further research is needed to clarify the changes in ruminal pH, feed intake patterns, and physiological responses after short-term fasting.

VFAs are the most abundant of the organic acids that determine ruminal pH [19]. In addition, shifts in molar proportions of VFAs, including a decrease in acetate and an increase in butyrate, are commonly observed in cattle fed an HC-diet [15, 23, 24]. In the present study, on Day −1, although total VFA changed significantly only in cattle fed the HC-diet, differences in the proportions of individual VFAs between cattle fed the HR- and HC-diets were consistent with findings from studies on the general characteristics of rumen fermentation in cattle consuming high-roughage and high-concentrate feed [15, 23, 24]. Similar trends were observed with regard to circadian changes in ruminal pH in the present study. Our results revealed that short-term fasting decreased ruminal VFA and NH₃-N concentrations in cattle fed the HR- and HC-diets, and VFA concentrations decreased more sharply in cattle fed the latter diet. In addition, cattle fed the HC-diet had a lower ruminal pH and higher VFA concentrations, consistent with results of previous studies that successfully induced subacute ruminal acidosis by feeding cattle an HC-diet [20, 24].

Severe feed restriction and complete feed deprivation negatively affect energy balance [5]. Fasting results in decreased plasma levels of insulin, GLU, and insulin-like growth factor-1 and increased NEFA in dairy cows [7, 18, 21] and beef cattle [3]. Reduced plasma leptin levels due to fasting have reported in prepubertal beef cattle [3, 6], but these levels recover when feeding is resumed in Holstein cows [8]. In the present study, no significant changes in GLU concentrations were observed in cattle fed both the HR- and HC-diets. However, NEFA levels increased significantly in cattle fed the HR-diet (at 2000) after fasting, and BHB concentrations decreased significantly in cattle fed the HC-diet (at 1400) compared to the levels at 0800. Therefore, based on the NEFA levels and BHB concentrations observed on Day 0, cattle may experience a negative energy balance even after a single episode of feed restriction. On the other hand, ruminal and blood components were not investigated on Day 1 in the present study, because we considered before the experiment that effects of only one time fasting on ruminal and blood metabolites might be limited. The ruminal pH on Day 1, however, revealed the different circadian pattern from Day −1 in the present study. Therefore, further study needs to clarify the effects of one time fasting on ruminal and blood metabolites.

In conclusion, a single morning fasting increased ruminal pH and blood NEFA levels and decreased ruminal VFA concentrations in cattle fed both an HR- and HC-diet. Our results indicate that a single episode of fasting is enough to disrupt ruminal fermentation, and these effects persist until the day after fasting. Therefore, special care might need to avoid negative effects of short-term fasting on ruminal fermentation, and to aid appearance of appetite in cattle after surgery with various diseases. Further research is needed to identify strategies for mitigating the negative effects of short-term fasting on rumen absorptive function [29, 30].

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