Variation of rumen bacterial diversity in steers after the beginning of grazing

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Introduction

Holstein heifers before or after puberty often are herded on public pastures in Japan. The herbage intake and rumen fermentation of grazing heifers that are not adapted for fresh herbage decreases due to a change of feed from stall-fed dried forage to fresh herbage. This limits their performance during the first several weeks on pasture. Thus, the feeding program such as supplementation before and after the beginning of grazing is important. An increase in ammonia concentration and a decline in fibre degradation in the rumen of a heifer (both of which occur simultaneously with low herbage intake and rumen fermentation) would be caused by the reduced capacity of various bacteria to produce peptides and degrade fibre (Oshio and Tahata 1981). This suggests that variation in rumen bacterial diversity plays an important role in herbage intake and rumen fermentation. However, less information is available on how bacterial diversity in heifers varies during the first few weeks of grazing. This information will provide the basis for designing nutritional management programs for heifers before and after the beginning of grazing. The objective of this study was to determine how herbage intake, digestibility, and rumen bacterial diversity vary in steers that have started grazing without adaptation for fresh herbage during the first 4 weeks after the beginning of grazing.

Materials and methods

This study was carried out at the NARO Institute of Livestock and Grassland Science, Tochigi, Japan (36°55'N, 139°55'E) from May 16 to July 12, 2011. Eight Holstein steers [average body weight (BW): 413 kg] fitted with cannula on their rumen were housed in individual pens from May 16 to June 13. Total mixed ration containing 60% grass silage, 11% corn silage, and 23% concentrate were offered to animals twice daily to meet 1.5 times their maintenance energy requirements. Steers were then grazed, without an adaptation period, on a perennial ryegrass (*Lolium perenne* L.)-dominant pasture (2 ha) divided into paddocks (approximately 0.08 ha). They were moved to a new paddock every 1 or 2 days. The herbage intake of the steers was estimated from fecal output and apparent digestibility on days 5-7 (wk 1), 12-14 (wk 2), 19-21 (wk 3), and 26-28 (wk 4) after the beginning of grazing. Rumen fluid samples were collected at 13:00 on the day before grazing (day -1) and days 1, 3, 7, 14, 21 and 28. Fecal output and apparent digestibility were estimated using chromic oxide and *n*-alkane, respectively. Total DNA from rumen fluid for bacterial diversity was extracted as described by Yu and Morrison (2004). For PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis of total bacteria, the V3 hypervariable region of the 16S rRNA gene was amplified using a universal primer set, to which a 40-nucleotide GC clamp was added. Comparison of PCR-DGGE profiles was performed with the BioNumerics software package using the UPGMA method. Some excised bands detected strongly in PCR-DGGE analysis were re-amplified by PCR, and their fragments cloned and sequenced. These sequences were identified using the NCBI BLAST service. Dry matter (DM) intake and digestibility data were analyzed using repeated-measures ANOVA with Tukey's *post hoc* test (SAS Inst. Inc., Cary, NC).

Results and discussion

Crude protein (CP) and neutral detergent fibre contents in the herbage ranged between 169 to 267 g/kg DM and between 411 to 543 g/kg DM, respectively. The DM intake was low in wk 1 (7.2 g DM/kg BW) and high in wk 4 (10.5 g DM/kg BW) (Fig. 1a, $P<0.05$). The DM digestibility was the lowest in wk 2 (0.56) and highest in wk 4 (0.69) (Fig. 1b, $P<0.05$). The negative daily gain, high serum non-esterified fatty acid (NEFA) concentrations and low short-chain fatty acid (SCFA) concentrations in the rumen of steers were detected until day 21 (Nakano *et al*. 2012), suggesting that grazing steers could not satisfy their energy requirements until day 21.

Comparing the PCR-DGGE profiles of each grazing steer from days -1 to 28, the rumen bacterial profiles of steer No. 1 had low similarity between days 21 and 28 (Fig. 2a). The similarity between days 21 and 28 was higher compared to the profiles between these days and the other days in steers No. 4 and 6 (Fig. 2b and c). These results indicate that rumen bacterial diversity may recover between days 21 and 28 after the beginning of

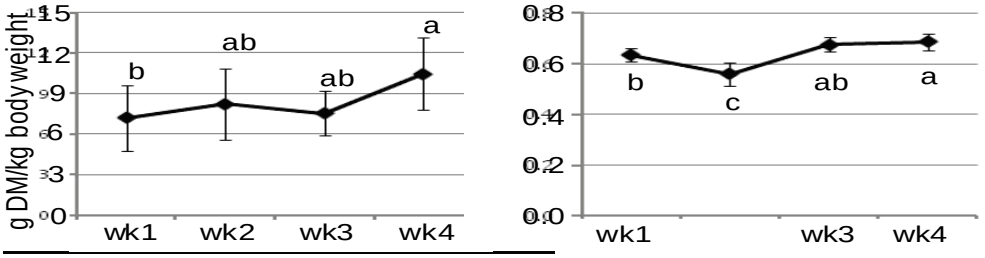

Figure 1. Variation of (a) dry matter (DM) intake and (b) digestibility over 4 weeks after the beginning of grazing. Error bars indicate the standard deviation. Mean values without common superscripts are significantly different at *P*<0.05.

Figure 2. PCR-denaturing gradient gel electrophoresis profiles generated from rumen bacterial 16S rRNA gene amplicons from (a) steer No. 1, (b) steer No. 4, and (c) steer No. 6, on days -1 (total mixed ration), 3, 7, 14, 21, and 28 after the beginning of grazing. Scale bar above each figure indicates percentage similarity coefficients.

grazing, although different tendencies in this regards were observed among the steers.

When PCR-DGGE profiles from steers on days 1 and 28, in which the nutritional condition of the steers would approximate recovery, were analyzed, these profiles formed distinct clusters based on sampling day. Some bands shared by all or most steers on day 28 in this analysis were identified as *Prevotella bryantii*, *Butyrivibrio fibrisolvens*, and *Prevotella ruminicolla*. *Prevotella* sp. is a major member of the rumen bacterial community. *B. fibrisolvens* was reported to be important in protein breakdown in pasture-grazed New Zealand cattle (Attwood and Reilly 1995). The pasture used in this experiment also had relatively high CP levels, similar to pastures in New Zealand. Therefore, these bacterial species, especially *B. fibrisolvens* may have an important role for transition from stall-fed dried forage to fresh herbage in the rumen of grazing steers after the beginning of grazing

Conclusion

The present study indicates that steers grazing on pasture without adaptation for fresh herbage need 3 or 4 weeks for recovery of nutritional status involving rumen bacterial diversity. The bands in PCR-DGGE analysis that were strongly detected on day 28 after the beginning of grazing were identified as *Prevotella* sp.and *Butyrivibrio* sp., which are both involved in protein breakdown. These bacterial species will be the key elements in the design of a feeding and supplementation program for heifers before and after the beginning of grazing.

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