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Effect of Plant-species Richness on Microbial Composition and Rumen Function

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Abstract

Plant diversity has been known to affect the productivity and long-term stability of grassland ecosystem, as well as the mineral balance in grazing cattle. However, there is little information on the relationship between plant diversity and livestock productivity traits such as rumen digestion and fermentation. Rumen bacteria play an essential role in the fermentation and digestion of cattle diet. In microbiology, a highly diverse microbial community is known to be able to respond quickly and flexibly to environmental changes. In fibrous diets-fed cattle, the rumen bacterial diversity is found to be high diverse, as plant-based fibers are rich in complex polysaccharides that enrich the microbial community. Based on the above information, we proposed the following hypothesis: complex fibrous composition of plants and high fiber intake by cattle in native pastures with greater plant species richness lead to high rumen bacterial diversity, which ensures stable fermentation and digestion, as well as flexibility toward rumen environmental changes. This year, we have investigated the rumen bacterial composition profile and rumen digestibility of cows grazing in two pastures with different plant species richness, using molecular biology techniques and in vitro incubation, respectively. Stability will be evaluated in terms of changes in bacterial composition and digestibility after a change in the feeding regime from pasture to barn. The authors believe that understanding the interaction between plant-species richness and livestock productivity will provide not only an evaluation of grassland ecosystem capability, but also the primary knowledge about the maintenance of stable rumen condition for livestock production.

A grassland ecosystem with high plant diversity has been expected to have multiple functions in livestock

production. Plant diversity has been known to affect the productivity and long-term stability of a grassland ecosystem (Tilman *et al.*, 2006) as well as plant nutritional characteristics as a feed (Mizuno *et al.*, 2014) and the mineral balance in grazing cattle (Mizuno *et al.*, 2011). However, there is little information on the relationship between plant diversity and livestock productivity traits, such as rumen digestion and fermentation, which are important for livestock production. Microbes, especially bacteria, are essential in rumen function. Bacteria play an essential role in the fermentation of carbohydrates into volatile fatty acids, the degradation of dietary protein, and the partial recapture of non-protein nitrogen as microbial cell protein for subsequent protein nutrition (Mohammed *et al.*, 2014). Therefore, it is necessary to determine rumen bacterial composition as well as its interaction with plant diversity and rumen function—digestion and fermentation—in cattle to evaluate a grassland ecosystem with high plant diversity with respect to livestock production.

A highly diverse microbial community is known to be able to respond quickly and flexibly to environmental changes (Miki, 2011). In rumen microbiology, the rumen bacterial diversity is found to be higher in forage-fed cattle than in high grain diet-fed cattle (Petri *et al.*, 2013). Plant-based fibrous diets are rich in complex polysaccharides (e.g., cellulose and hemicellulose), lignin, and the other phenolic compounds. Fibrous feed particles composed of these compounds are colonized exclusively by closely adherent bacteria (Pitta *et al.*, 2010). Some bacteria specialize in the transformation of plant lignin and the other phenolic compounds and in the formation of secondary substrates, such as cinnamic acid, which enhance polysaccharide hydrolysis by the other members of the biofilm (Larue *et al.*, 2005). These

adherent bacteria are often associated with consortia of secondary bacterial colonizers (Pitta *et al.*, 2010). Furthermore, the long rumen retention time of fibrous diet facilitates the development of a more complex array of diet and intermediary substrates to support a more diverse bacterial community (Pitta *et al.*, 2010). This information suggests that increases in the fibrous nature of the feed particles and the fiber intake of cattle result in greater diversity in the rumen bacterial population, leading to a stable rumen condition that responds quickly and flexibly to rumen environmental changes.

Generally, more plant species are observed in a native pasture compared to those in a sown pasture. Mizuno *et al.* (2012) reported that the number of plant species in pasture vegetation and herbage ingested by cows grazing on a native pasture was higher than that ingested by cows grazing on a sown pasture. The fibrous compound content of herbage ingested by cows grazing on a native pasture is also higher than that of herbage collected on a sown pasture (Nakano *et al.*, 2007; NARO, 2009); seasonal changes in contents of crude protein and neutral detergent fiber of herbage ingested by cows grazing on a native pasture were relatively small (Nakano *et al.*, 2015). The nutritional status (Nakano *et al.*, 2015) and mineral balances (Mizuno *et al.*, 2012) of cows grazing on a native pasture were relatively good, although scant dry matter and energy intake was detected in the fall (Nakano *et al.*, 2015). Based on this information, we hypothesized that ingestion by cattle of a complex fibrous composition of plants owing to the foraging of many fibrous plant species along with the high fiber intake in the native pasture with greater plant-species richness led to high rumen bacterial diversity. This ensures stable rumen function and productivity, flexibility towards rumen environmental changes, and good nutritional status of cattle own.

In preliminary research conducted at the Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan (38° 44' N, 140° 15' E, 300–600 m in elevation) in 2015, the rumen bacterial profiles of cows grazing on two native pastures with medium or high plant-species richness were compared using PCR-denaturing gradient gel electrophoresis (DGGE) of rumen bacterial 16S rRNA gene amplicons. Many bands were visually detected in the profiles of cows grazing on pasture with high plant-species richness compared with

those grazing on pasture with medium plant-species richness (Fig. 1). This result suggested that the rumen bacterial population in cows grazing on a pasture with high plant-species richness might be more diverse and characteristic than that observed in cows grazing on a pasture with medium plant-species richness.

The PCR-DGGE method used in this preliminary research allows the rapid screening of bacterial populations and visualization of PCR products representing predominant rumen bacterial communities (Hume *et al.*, 2003). However, this method has to be used in combination with other analyses to identify the bacterial species and to define a detailed population and community structure. Recently, some researchers have studied rumen bacterial populations of cattle fed with high grain diet, high forage diet, silage, and fresh herbage by using next-generation sequencing approaches based on pyrosequencing (Pitta *et al.*, 2010; Mohammed *et al.*, 2014). Petri *et al.* (2013) determined the changes in rumen microbial populations as a result of acidotic inducing diets within individual animals, that have not been well elucidated by using DGGE method, by using 454-pyrosequencing. This approach enables us a new discovery through cost-effective sequence throughput in a relatively short time (Morozova and Marra, 2008). Furthermore, several studies have identified associations between particular rumen bacterial groups and fermentation products in cattle (Hernandez-Sanabria *et al.*, 2010), indicating that the focus in the recent rumen bacterial studies have shifted from determining “who is there” (population) towards understanding “what are they doing” (their mechanisms of action) (Li *et al.*, 2015). The focused correlations among microbial communities in the rumen ecosystems of cattle (Kittelman *et al.*, 2013) and between bacterial communities and metabolic phenotypes of sika deer (Li *et al.*, 2015) have been described visually by using co-occurrence network analysis. Co-occurrence network analysis is generally used to provide a graphic visualization of potential relationships between people, organizations, concepts, or other entities represented within the available information (Yasuda, 1997). In rumen microbiology, this analysis is employed for understanding the metabolic networks between rumen-inhabiting microbial groups and resolving their individual contributions to overall rumen functioning (Kittelman *et al.*, 2013). This approach may provide new clues about complex interactions among rumen

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bacterial diversity, rumen function, and its stability in cattle grazing on species-rich pastures.

Using next generation sequencing and co-occurrence network analysis, the objectives of this study were to 1) investigate differences in the fibrous composition of ingested herbage, rumen retention time, and bacterial composition of cattle grazing on pastures with high or low plant-species richness; 2) explore the associations among rumen bacterial diversity, rumen function (rumen digestibility), and productivity (fermentation characteristics and amount of microbial protein synthesis); 3) explore the relationship between rumen bacterial diversity and stabilities of rumen digestion and fermentation; and 4) test the hypothesis that rumen bacterial diversity and rumen function and productivity are related to the plant-species richness in a pasture.

The study has conducted from May 2016 at the Field Science Center, Graduate School of Agricultural Science, Tohoku University, Japan (38° 44' N, 140° 15' E, 300–600 m in elevation). The typical rumen bacterial composition profile and rumen digestibility of cows grazing in two pastures with high (native pasture) or low (sown pasture) plant-species richness have been investigated using next-generation sequencing and *in vitro* incubation, respectively. Stability will be evaluated in terms of changes in bacterial composition and digestibility after a change in the feeding regime from pasture to barn. The authors have collected rumen fluid, urea, and blood samples

from cows grazing on native and sown pastures, and has analyzed elements associated with rumen bacterial diversity, rumen fermentation, bacterial cell protein synthesis, and nutritional status. The authors hope to obtain interesting discoveries and discuss with you in other opportunities.

Recently, in the dairy and beef production industry, rumen acidosis is increasingly recognized as a significant disorder. Rumen acidosis results from extreme fermentation due to feeding of high grain and low roughage diets. This extreme fermentation leads to an increase in acid and a decline in pH in the rumen, which alters the activity and abundance of many bacterial species (Petri *et al.*, 2013). Rumen fermentation and bacterial population during rumen acidosis have been extensively studied (Petri *et al.*, 2013; Mao *et al.*, 2013). Nevertheless, it is also necessary to reveal the stable rumen condition—bacterial composition and rumen function—that can adapt flexibly toward these rapid changes in feed. An understanding of the interaction between plant-species richness and livestock productivity would lead to an understanding of the factors responsible for stable rumen condition. The authors believe that understanding the interaction between plant-species richness and livestock productivity will provide not only an evaluation of grassland ecosystem capability, but also primary knowledge about the maintenance of stable rumen condition for livestock production.

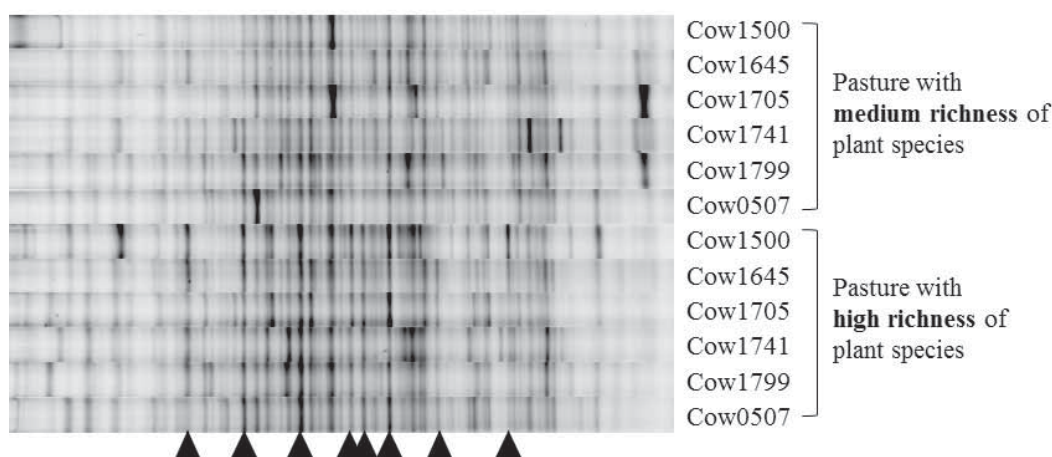


Fig. 1. Denaturing gradient gel electrophoresis (DGGE) profiles of rumen bacterial 16S rRNA gene amplicons from cows grazing on two native pastures with medium or high plant-species richness in the preliminary research conducted in 2015. The image was normalized using the DGGE Marker II (Nippongenem Toyama, Japan) and Bio Numerics software, version 5.0 (Applied Maths, Austin, TS). Filled triangles (▲) under the figure indicate the bands that were detected strongly or characteristically in pasture with high plant-species richness.

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References

- National Agriculture and Food Research Organization (NARO) (2009) Standard tables of feed composition in Japan. Japan Livestock Industry Association, Tokyo, Japan. (in Japanese)
- Hernandez-Sanabria, E., L.L. Guan, L.A. Goonewardene, M. Li, D.F. Mujibi, P. Stothard, S.S. Moore and M.C. Leon-Quintero (2010) Correlation of particular bacterial PCR-denaturing gradient gel electrophoresis patterns with bovine ruminal fermentation parameters and feed efficiency traits. *Appl Environ Microbiol*, 76: 6338–6350.
- Hume, M.E., L.F. Kubena, T.S. Edrington, C.J. Donskey, R.W. Moore, S.C. Ricke and D.J. Nisbet (2003) Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. *Poult Sci*, 82: 1100–1107.
- Kittlmann, S., H. Seedorf, W.A. Walters, J.C. Clemente, R. Knight, J.I. Gordon and P.H. Janssen (2013) Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal, and eukaryotic microorganisms in rumen microbial communities. *PLoS ONE*, 8: e47879.
- Larue, R., Z. Yu, V.A. Parisi, A.R. Egan, and M. Morrison (2005) Novel microbial diversity adherent to plant biomass in the herbivore gastrointestinal tract, as revealed by ribosomal intergenic spacer analysis and rrs gene sequencing. *Environ Microbiol*, 7: 530–543.
- Li, Z., A.G. Wright, H. Liu, K. Bao, T. Zhang, K. Wang, X. Cui, F. Yang, Z. Zhang, and G. Li (2015) Bacterial community composition and fermentation patterns in the rumen of sika Deer (*Cervus nippon*) fed three different diets. *Microb Ecol*, 69: 307–318.
- Mao, S.Y., R.Y. Zhang, D.S. Wang, and W.Y. Zhu (2013) Impact of subacute ruminal acidosis (SARA) adaptation on rumen microbiota in dairy cattle using pyrosequencing. *Anaerobe*, 24: 12–19.
- Miki, T (2011) Microbial Population Biology. In *Microbial Ecology*, T. Ohsono and M. Kagami (Eds.), pp. 222–237, Ecological Society of Japan, Tokyo. (in Japanese)
- Mizuno, H., M. Nakano, M. Tohno, K. Kimura and S. Ogura (2014) Rumen conditions of cattle and sheep grazed in pasture with different plant species richness. Knowledge innovation of grassland science and sustainable development of grassland agriculture. Proceedings of the 5th China-Japan-Korea Grassland Conference, p. 203.
- Mizuno, H., Y. Yoshihara, T. Inoue, K. Kimura, S. Tanaka, S. Sato and S. Ogura (2012) The effect of species richness of vegetation on mineral condition of grazing cattle in a Japanese alpine pasture. Evolution and future challenges of grasslands and grassland agriculture in the East Asia. Proceedings of the 4th Japan-China-Korea Grassland Conference, pp. 242–243.
- Mohammed, R., G.E. Brink, D.M. Stevenson, A. P. Neumann, K.A. Beauchemin, G. Suem and P.J. Weiner (2014) Bacterial communities in the rumen of Holstein heifers differ when fed orchardgrass as pasture vs. hay. *Front Microbiol*, 5: 1–11.
- Morozova, O. and A.M. Marra (2008) Applications of next-generation sequencing technologies in functional genomics. *Genomics*, 92: 255–264.
- Nagaraja, T.G. and K.F. Lechtenberg (2007) Acidosis in feedlot cattle. *Veterinary Clinics of North America. Food Anim Pract*, 23: 333–350.
- Nakano, M., M. Yayota and S. Ohtani (2015) Nutrient sufficiency in beef cows grazing on a dwarf bamboo-dominated forestland pasture in Japan. *Anim Sci J*, 86: 689–697.
- Nakano, M., M. Yayota, J. Karashima and S. Ohtani (2007) Seasonal variation of nutrient intake and digestibility of forage in beef cows grazed on a dwarf bamboo (*Pleioblastus argenteostriatus f. glaber*) dominant pasture. *Grassl Sci*, 53: 69–77.
- Petri, M.R., T. Schwaiger, G.B. Penner, K.A. Beauchemin, R.J. Forester, J.J. McKinnon and T.A. McAllister. (2013) Characterization of the core rumen microbiome in cattle during transition from forage to concentrate as well as during and after an acidotic challenge. *PLoS ONE*, 8: e83424.
- Petri, R.M., R.J. Forster, W. Yang, J.J. McKinnon and

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- T.A. McAllister (2012) Characterization of rumen bacterial diversity and fermentation parameters in concentrate fed cattle with and without forage. *J Appl Microbiol*, 112: 1152–1162.
- Pitta, D.W., W.E. Pinchak, S.E. Dowd, J. Osterstock, W. Gontcharova, E. Youn, K. Dorton, I. Yoon, B.R. Min, J.D. Fulford, T.A. Wickersham and D.P. Malinowski (2010) Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. *Microb Ecol*, 59: 511–522.
- Tilman, D., P.B. Reich and J.M. Knops (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, 441: 629–632.
- Yasuda, Y (1997) Network analysis. Y. Yasuda (Ed.), pp. 1-256, Shinyosya, Tokyo, Japan.