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The XXIV International Grassland Congress / XI International Rangeland Congress (Sustainable Use of Grassland and Rangeland Resources for Improved Livelihoods) takes place virtually from October 25 through October 29, 2021.

Proceedings edited by the National Organizing Committee of 2021 IGC/IRC Congress Published by the Kenya Agricultural and Livestock Research Organization

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# Polyphenol concentration of native plant species, and its effect on blood antioxidant capacity in grazing cattle in a species-rich vegetation in Japan

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Key words: catechin; diet composition; grazing cattle; plant species diversity; polyphenols

## Abstract

It is known that polyphenols in plants have a high antioxidant capacity. However, there is scarce information on its concentration in native plants and the effect of antioxidant capacity to grazing animals. In this study, polyphenol concentration of available plants was investigated in a species-rich grazing area in north-eastern district of Japan. In addition, polyphenol concentration in blood serum of grazing cattle was also measured. Eight beef cows grazed in a grazing area (hill pasture 3.1 ha; forest 16.9 ha) from late spring to mid-autumn (142 days), and four cows among the eight grazed at an orchardgrass pasture in mid-summer (10 days). During the grazing period, foraging behaviour was directly observed, and plant species proportion in ingesta and residence time in the hill pasture and the forest were measured. Based on these results, the top 10–13 species were hand-clipped by mimicking foraging manner of cows, and total polyphenol and catechin concentration were measured. Blood samples of cows were also collected during the grazing period, and potential antioxidant [PAO] and serum total antioxidant status [STAS] were analysed. The cows ingested 17-32 plant species in the hill pasture, and 53-73 species in the forest. In contrast, the cows ingested mainly orchardgrass in the sown pasture in mid-summer. Total polyphenol and catechin concentration were higher in tree leaves (112.8-209.3 g/kg DM, 0.081–6.250 g/kg DM) than monocots (11.9–34.0 g/kg DM, 0–0.159g/kg DM). However, those concentration in ingesta of the cows were low throughout the seasons (35.0-56.9 g/kg DM, 0.108-0.467 g/kg DM), as in the sown pasture (26.2 g/kg DM, 0.158 g/kg DM), due to high proportion of monocots in ingesta at the hill pasture (67–75%). PAO (373.4–455.8 µ mol/L) and STAS (769.8–910.0 µ mol/L) of the cows were almost constant throughout the seasons.

## Introduction

Recently, much attention has been paid for functions of ecological diversity in grasslands and rangelands. Our research works revealed that grazing animals encounter and consume a wide range of plant species in diverse, species-rich pastures (Ogura 2011). Plant species-richness likely affects the amount and proportion of dietary nutrients for grazing animals, because the nutrient composition of plants varies among species (Ohlson and Staaland 2001), and access to a wider range of species provides foraging animals a wider range of choices. It is known that shrubs and trees of several types have high total antioxidant capacity and forest grazing increase plasma antioxidative activity of cattle (Haga et al. 2014; 2016). Polyphenols in plants have a high antioxidant capacity, which potentially improve antioxidant capacity of grazing animals (Surai 2014). However, there is scarce information on its concentration in native plants and the effect of antioxidant capacity to grazing animals.

Therefore, in this study, polyphenol concentration of available plants was investigated in a species-rich grazing area in north-eastern district of Japan. In addition, polyphenol concentration in blood serum of grazing cattle was also measured.

## Methods and Study Site

The experiment was conducted at the Field Science Center, Graduate School of Agricultural Science, Tohoku University (Osaki, Miyagi, Japan, 38°N, 140°E). Eight beef cows grazed in a hill grazing area (hill pasture 3.1 ha; forest 16.9 ha) from late spring to mid-autumn (142 days), and four cows among the eight grazed at a sown pasture dominated by orchardgrass (*Dactylis glomerata* L.) in mid-summer (10 days). In both grazing area, the cows were allowed to access to water and mineral salt block in *ad limitum*. All the animal experimental procedures were approved by the Committee for Animal Experiment and Welfare of Tohoku University (2017AgA-025-1).

Plant species ingested by the cows were visually identified and recorded by 2-hrs observation of the eight animals, at 30-second intervals during foraging bout in the morning and evening, in 12–20 June (early summer), 26 July–2 August (mid-summer) and 20–28 September (early autumn), 2018. Vegetation type (*i.e.*, pasture or forest) of the locations where the cows foraged was also recorded. From these data, plant species

proportion in ingesta was calculated for each plant species. A GPS device and a motion sensor were set to all the cows to record their grazing time in pasture and forest.

Based on these results, the top 10–13 species were chosen and hand-clipped by mimicking foraging manner of cows, and total polyphenol and catechin concentration were analysed by Folin-Ciocalteu method and high-performance liquid chromatography, respectively. Blood samples of the cows were also collected seven times at 9–33 days interval from jugular vein during the grazing period, and potential antioxidant (PAO) and serum total antioxidant status (STAS) were analysed by a colorimetric method.

#### Results

## Plant Species Ingested by Grazing Cows

In the hill grazing area, the cows ingested 17–32 plant species (e.g., Anthoxanthum odoratum L., Carex albata Boott. and Rumex acetosella L.) in the hill pasture, and 53–73 species (mainly tree leaves of Carpinus laxiflora (Sieb. et Zucc.) Blume, Acer rufinerve Siebold et Zucc. and Viburnum dilatatum Thunb.) in the forest. The cows spent 33.4% (2.8 h/day) and 23.5% (2.2 h/day) of foraging time in the forest. The proportion of tree species in foraging was 11-20%. In contrast, the cows ingested mainly orchardgrass in the sown pasture in mid-summer.

#### Polyphenols and Catechin Concentration in Plants and Ingesta of the Cows

Total polyphenol concentration of plants was higher in tree leaves (112.8–209.3 g/kg DM) than monocotyledons (11.9–34.0 g/kg DM) and forbs (40.6–109.2 g/kg DM). Similarly, catechin concentration of plants was also higher in tree leaves (0.081–6.250 g/kg DM) than monocotyledons (0–0.159g/kg DM) and forbs (0–2.948 g/kg DM). However, those concentration in ingesta of the cows were low (35.0–56.9 g/kg DM, 0.108–0.467 g/kg DM) as same as in the sown pasture (26.2 g/kg DM, 0.158 g/kg DM) throughout the grazing seasons, due to high proportion of monocots in ingesta at the hill pasture (67–75%).

#### Antioxidative activity of Serum of the Cows

Serum PAO and STAS of the cows ranged 373.4–455.8  $\mu$  mol/L and 769.8–910.0  $\mu$  mol/L, respectively. Both antioxidative activity did not vary throughout the seasons.

# Discussion

The number of plant species appearing in the pasture was higher in the forest than in the pasture, and the number of plants ingested by cattle was also higher in the forest in both seasons. However, the daily foraging time was longer in the pasture throughout the season. Therefore, it is likely that the foraging time is longer in pastures under such pasture-forest combined area.

In this study, the total polyphenol content was higher in tree leaves than in monocotyledons. This finding is similar to the reports of Haga *et al.* (2014; 2016) which indicated that the antioxidant capacity of tree leaves is higher than that of monocotyledons. These findings suggest that bovine foraging on woody plants in addition to grasses under diverse vegetative conditions potentially result in a high intake of total polyphenols. However, in this study, the concentration of total polyphenols and catechin in ingesta of the cows were almost same as in the sown pasture. Haga *et al.* (2016) showed that the plasma antioxidant capacity of grazing cattle in forestland increased when the proportion of tree species consumed was about 30-55%. The proportion is higher than in this study, suggesting that more than 30% of foraging proportion of tree species to obtain a significant increase in plasma antioxidant capacity in bovine grazing in forestlands.

In addition, total polyphenol intake was significantly higher in the mountain grazing area, but there was no difference in serum antioxidant capacity (STAS, PAO) of the grazing cows between the hill grazing area and the sown pasture; the difference in the amount of polyphenol intake due to grazing under different vegetation did not affect the serum antioxidant capacity of grazing cattle. This may be due to the fact that both grazing areas did not consume enough polyphenols and catechin to affect the serum antioxidant capacity of cattle, or that the effect of polyphenol intake on serum antioxidant capacity was small.

In conclusions, this study indicated that tree species have high total polyphenols and catechin concentration than herbaceous plant species, particularly monocotyledons, throughout the grazing season. However, serum antioxidant capacity of grazing cows does not increase if the proportion of tree leaves in ingesta is low (<20%).

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