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In this study, *in vitr*o digestion experiment was conducted to examine the effect of plant species richness in diet on ruminal digestion of plants in grazing cattle. One grass (*Dactylis glomerata*), two forbs (*Trifolium repens* and *Rumex acetosella*) and one tree species (*Acer rufinerve*) were collected from a grazing paddock of Field Science Center, Tohoku University, Japan, in mid-summer. These samples were air-dried and grounded (0.5 mm screen). Ruminal fluid was collected by using oral catheter from cows grazed on a mountainous area (pasture + forest; PF) and an orchard grass sown pasture (SP), and filtrated by using duplicate gauze. These rumen fluid samples were used as inoculum of digestion experiment after dilution with McDougall artificial saliva (1:4) and saturated with CO_2. The plant samples (0.5 g) and the rumen inoculum (50 mL) were incubated for 0, 24, 48 h at 39°C. Residue was collected by filter paper and dried at 105°C for 16 h to determine dry matter digestibility (DMD). The effect of plant species, ruminal fluid and these interaction were all significant (P<0.05) after 24–48 h incubation. In *A. rufinerve*, DMD was significantly lower than in other plant species for all incubation time (P<0.01). DMD of forbs was significantly different between ruminal fluid treatment (P<0.05); DMD of *R. acetosella* was PF>SP after 24 h incubation, and that of *T. repens* was SP>PF after 24 h but PF>SP after 48 h incubation. In contrast, there was no significant difference in *D. glomerata.* The results suggest that species richness of diet affect ruminal digestibility of plants.