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【Short Report】

Changes in Nutritive Value of Italian Ryegrass (*Lolium multiflorum* Lam.) during Overwintering Period

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Italian ryegrass (*Lolium multiflorum* Lam) is the most popular winter annual grass in Japan and has been cultivated for hay or silage. Recently, its use for winter grazing has attracted attention because the reduction of the labor involved in animal management is in demand. In the western region of the main island of Japan, grazing systems are considerably different between summer and winter grazing. While active growth of the grass supplies the forage to livestock in summer, the main forage in winter is the grass that has grown during autumn because low temperatures reduce the growth of Italian ryegrass in winter. Therefore, pasture management methods such as non-grazing during autumn are required to accumulate sufficient above-ground biomass for winter grazing.

In addition to the growth reduction, low temperatures in winter are known to induce a phenomenon called "cold acclimation" in plants. Cold acclimation is a physiological response of plants that enables them to withstand low temperatures. It includes many processes such as the accumulation of metabolites, modification of membranes and increase in antioxidants (Xin and Browse, 2000; Cai et al., 2004). In ryegrasses, the accumulation of water-soluble carbohydrates, such as fructan, has been reported (Pollock and Jones, 1979; Tamura et al., 1985). Water-soluble carbohydrates are included among the non-fiber carbohydrates (NFC); they function as a nutrition source for animals. Therefore, an increase in the content of water-soluble carbohydrates induced by cold acclimation may affect the nutritive value of Italian ryegrass. Previous reports have examined the role of cold acclimation only with regard to the winter survival or the spring growth of ryegrasses (Pollock and Jones, 1979; Tamura et al., 1985). However, the effects of cold acclimation on the nutritive value of Italian ryegrass, such as total digestible nutrients (TDN), remain unclear.

In the present study, we explored the changes in

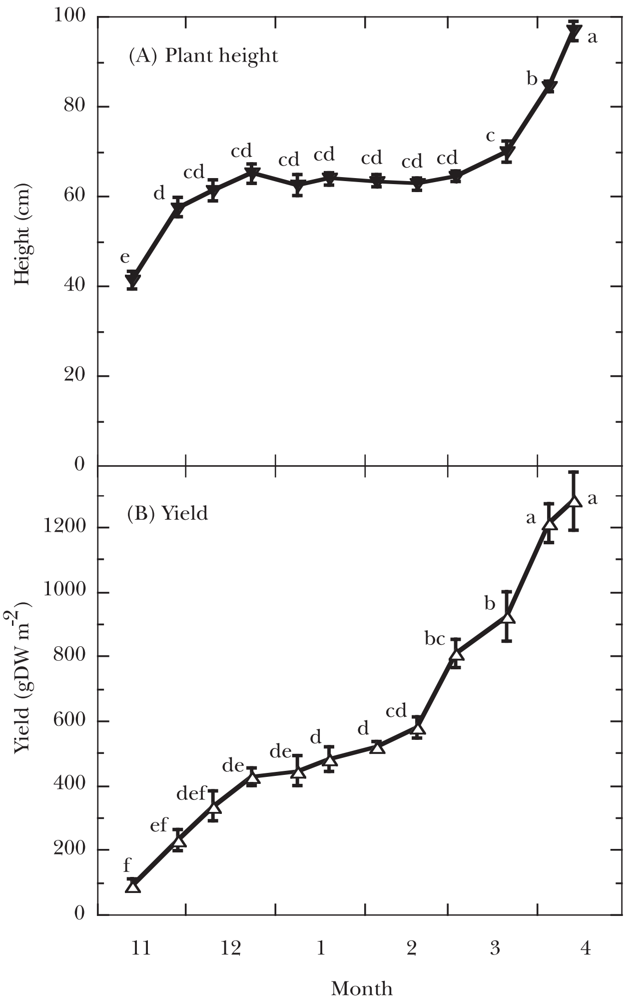

Fig. 1. Plant height (A) and yield (B) of Italian ryegrass.
For plant height (A), data are means ± standard error (SE) with 15 replicates.
For yield (B), data are means ± SE with 3 replicates.
Data followed by the same letters are not significantly different (P<0.05) according to Tukey's test.

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Abbreviations : ADF, acid detergent fiber; DW, dry weight; EE, ether extract; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates; TDN, total digestible nutrient.

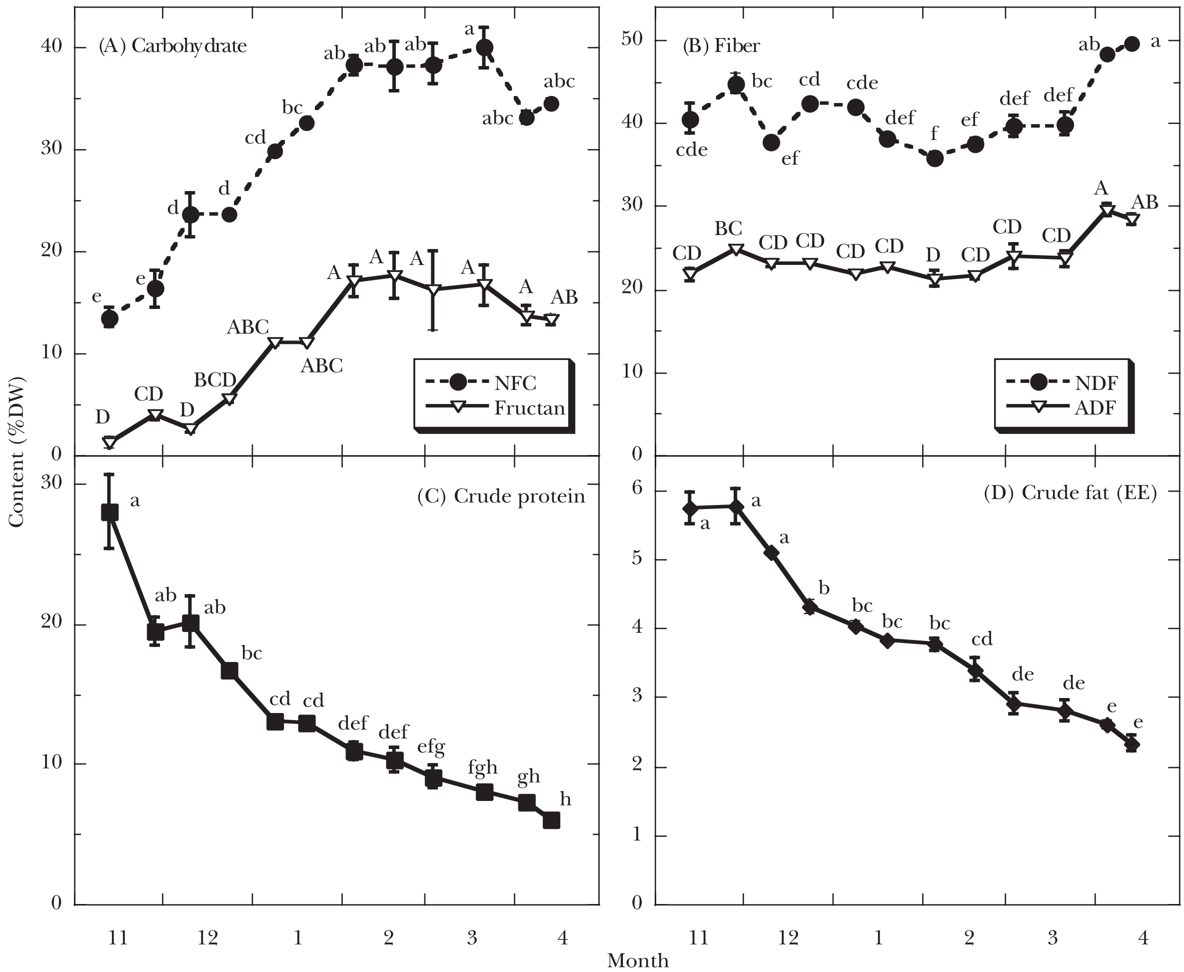

Fig. 2. Contents of nutritive components in Italian ryegrass.
(A) Non-fiber carbohydrates (NFC) and fructan. (B) Neutral detergent fiber (NDF) and acid detergent fiber (ADF). (C) Crude protein. (D) Crude fat (ether extract [EE]).
Data are means ± SE with 3 replicates.
Data followed by the same letters are not significantly different ($P < 0.05$) according to Tukey's test.

the nutritive components of Italian ryegrass during the overwintering period in order to examine the effects of cold acclimation on its nutritive value.

Materials and Methods

The experiment was conducted at the Ohda Research Station of the National Agricultural Research Center for Western Region (132°30'E, 35°10'N) during the winter of 2006–2007. In the present study, we used Italian ryegrass (*Lolium multiflorum* Lam. cv. Nagahahikari); this cultivar is a tetraploid, medium-maturing cultivar. Seeds were broadcast in the experimental field (300 m^2) on 3 October 2006 at the rate of 3 g m^{-2}. The field was fertilized with 2 kg m^{-2} of fermented cattle manure, 45 g m^{-2} of ground dolomitic limestone and 67 g m^{-2} of chemical compound fertilizer (N : P : K = 15 : 15 : 15). All the fertilizers were applied as basal dressing. During the experiment, the sward was not defoliated.

The meteorological data recorded at the nearest automated weather station (AMeDAS Ohda (132° 30.1'E, 35°11.5'N), approximately 3 km north from the experimental field) during the experiment were as follows: mean temperature: 10.0°C, lowest temperature: –1.0°C (recorded on 26 February), highest temperature: 27.6°C (recorded on 10 October) and rainfall: 576 mm/194 d.

The sward was sampled every 2 wks from 13 November 2006 to 14 April 2007; the total number of samplings was 12. Heading of Italian ryegrass was observed on 23 April 2007, after the final sampling. Three plots (0.5 m^2 each) were randomly selected, and the shoots of Italian ryegrass in the plot were harvested at the ground surface. After the heights of 5 randomly selected plants in each plot were measured, the samples were dried at 60°C for 72 hrs and weighed. The dried samples were ground to pass a 1-mm screen and stored at room temperature until chemical analysis. An analysis of the nutritive components was conducted according to the methods of the Society

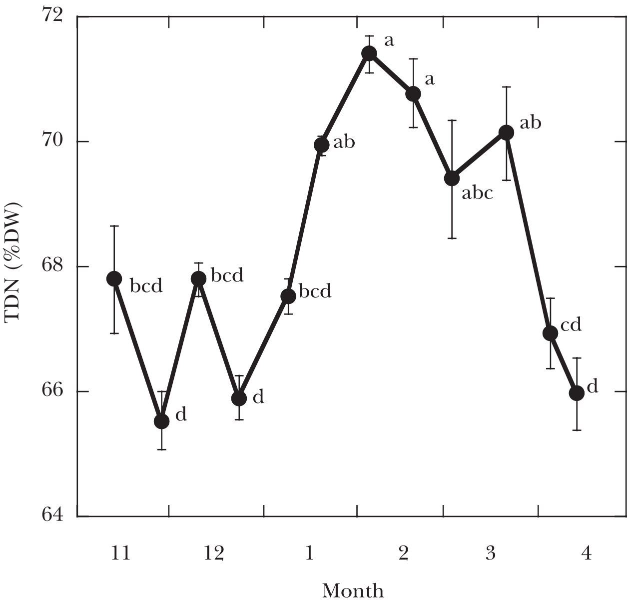

Fig. 3. Estimated total digestible nutrient (TDN) value.
TDN value was calculated using the equation of the National Research Council (2001).
Data are means ± SE with 3 replicates.
Data followed by the same letters are not significantly different ($P<0.05$) according to Tukey's test.

for Self-Supplying Feed Quality Assessment (2001). Fructan content was determined using a Fructan HK assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Total digestible nutrient (TDN) value was estimated using the equation of the National Research Council (2001):

$$\text{TDN}(\%\,\text{DW}) = \text{tdNFC} + \text{tdCP} + (\text{tdFA} \times 2.25) + \text{tdNDF} - 7,$$
$$\text{tdNFC} = 0.98 \times [100 - \{(\text{NDF} - \text{NDICP}) + \text{CP} + \text{EE} + \text{Ash}\}],$$
$$\text{tdCP} = \text{CP} \times \exp(-1.2 \times \text{ADICP}/\text{CP}),$$
$$\text{tdFA} = 0.94 \times (\text{EE} - 1) \times 2.8,$$
$$\text{tdNDF} = 0.75 \times \{(\text{NDF} - \text{NDICP}) - \text{ADL}\} \times [1 - \{\text{ADL}/(\text{NDF} - \text{NDICP})\}^{0.667}],$$

where, td indicates “truly digestible”; NFC, non-fiber carbohydrates; CP, crude protein; FA, fatty acids; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein; EE, ether extract; ADICP, acid detergent insoluble crude protein; ADL, acid detergent lignin.

Results and Discussion

During winter, the growth of Italian ryegrass was suppressed (Fig. 1). The plant height increased until late November, remained constant until late March and then increased again in April (Fig. 1A). The yield continued increasing during the experiment, but the increase was reduced from late December to late February (Fig. 1B). These results confirm that low temperature during winter reduces the growth of Italian ryegrass in the western region of the main island of Japan, and the main forage for winter grazing is the above-ground biomass accumulated during autumn.

Fructan accumulated while the shoot elongation was arrested (Figs. 1A and 2A). The content of fructan remained low until early December, increased to reach a maximum value in February (17.6% DW), and then slightly decreased in April (Fig. 2A). These trends were similar to those reported by previous studies with perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (Pollock and Jones, 1979; Tamura et al., 1985).

The accumulation of fructan affected the content of non-fiber carbohydrates (NFC). The content of NFC increased during winter, and the changes in NFC content were associated with the changes in fructan content (Fig. 2A). The difference between the maximum (22 March) and minimum contents (13 November) of NFC was 26.5% DW, and the difference in fructan content between these days was 15.5% DW, which was approximately 58% of that of NFC. These results indicate that the accumulation of fructan greatly contributes to the increase in NFC. Tamura et al. (1985) have reported that the degree of fructan accumulation induced by cold acclimation varies among the cultivars of Italian ryegrass. Therefore, the increase in NFC content during winter may also vary among the cultivars.

Changes in the content of the other nutritive components differed from the changes in NFC content. The content of neutral detergent fiber (NDF) fluctuated between 35 and 45% DW during winter and then increased in April (Fig. 2B). The content of acid detergent fiber (ADF) remained almost constant during winter and then increased in April (Fig. 2B). The contents of crude protein and crude fat (ether extract) constantly decreased during the experiment (Fig. 2C, D).

To evaluate the changes in the nutritive value of Italian ryegrass synthetically, we calculated the total digestible nutrient (TDN) value on the basis of the nutritive components of the grass (Fig. 3). The TDN value fluctuated between 65 and 68% DW until early January, increased to reach a maximum value in early February (71.4% DW), and then decreased in April. Therefore, the accumulation of NFC during winter could increase the TDN value, although the contents of crude protein and crude fat decreased (Figs. 2 and 3).

These results indicate that the nutritive value of Italian ryegrass significantly changes during the overwintering period as a result of cold acclimation. However, the effects of these changes on the livestock growth and/or the quality of animal products remain unclear. Therefore, further studies regarding livestock production during winter grazing are required.

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