Non-Structural Carbohydrates in Orchard Grass

By KAZUO SUGAWARA

Grassland Research Laboratory, Faculty of Agriculture, Tohoku University
(Narugo, Miyagi, 989-67 Japan)

Glucose, fructose and sucrose (free sugars), and starch and fructosans (poly-saccharides) are the predominant non-structural carbohydrates (NSC)[5] in grasses. These are important in perennial grasses as reserve materials for survival over cold winter, growth initiation in early spring, and regrowth after grazing and mowing practices. The reserve carbohydrates are mainly stored in stem base including corm, storon and rhizome, and root of plant. They are used by the plant itself as energy sources and substrates for the formation of new tissues when photosynthesis of the plant is insufficient to satisfy their demand. The levels of reserve carbohydrates[4,6,11] are different among the species and organs of grasses, and vary with the growth stage. They are also influenced by culture conditions such as temperature, soil moisture and use of fertilizer.

The perennial grasses are divided into two groups[1,3] based on the difference of reserve carbohydrate composition. The grasses (subfamily Festucoidea) of temperate-origin store predominantly sucrose and fructosans, whereas those (subfamilies Eragrostoidea, Panicoidea and Bambsoidea) of tropical and subtropical-origin store sucrose and starch.

The present paper presents a review on the NSC of orchard grass, which is one of fructosan accumulators and commonly cultivated forage grasses in Japan. The studies were carried out at Kawatabi farm of Tohoku University in the northern Japan.

Polymerization of fructosan under low temperature[7]

The fructosan in grasses is β-2, 6 linked D-fructo-franose polymers (levan type)[5] and consists of many fructose polymers of different degrees of polymerization. Orchard grass accumulates a large quantity of highly polymelized fructosans in its stubbles and roots, with a decline of temperature in autumn. Sometimes, the grass is managed with grazing or mowing in this season, and is obliged to consume a reserve food for wintering.

Table 1 shows a typical pattern of the quantitative NSC changes in stubbles after cutting in early October in the northern Japan. The average daily temperature was about 10°C in this season. NSC was about 26% of dry matter at the cutting time on Oct. 4. Then it decreased to 13.4% on Oct. 13. Thereafter the NSC concentration took a turn for increase and went up to the maximum level of 32% on Nov. 19 (5°C in temperature). Further increase of NSC concentration in the grass was not recorded although temperature decreased to below 0°C on Dec. 23. These changes of the NSC level are mainly attributed to amount of the fructosan fractions. The NSC in roots also showed a similar change to that in stubbles, but the extent

Table 1. Variation of sugar and fructosan concentrations* in stubbles of orchard grass after cutting

Sampling dates		Total sugar	Fructosan	Total NSC
Oct.	4	2.15%	23.83%	25.98%
	6	1.63	20.55	22.18
	9	2.23	20.08	22.31
	13	2.11	11.33	13.44
	23	1.88	13.99	15.87
Nov.	19	2.53	29.30	31.83
Dec.	23	3.88	27.19	31.07

* % of dry weight

of fluctuation was not so much as that in stubbles.

Fructosans in plant tissues are possibly separated from free sugars with 85% (v/v) ethanol, which extracts the latter. Then fractosans remaining in the tissues are able to be fractionated into several groups based upon the degrees of polymerization (DP) of fructose with a graded series of ethanol concentration. The short-chain fructosans are extracted with the ethanol solution of high concentration and the long-chain fructosans are extracted with the ethanol solution of successively lower concentrations.

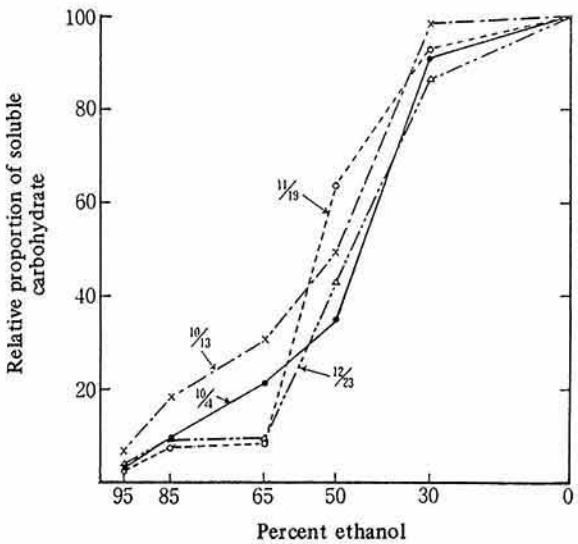

Fig. 1. Relative proportion of NSC extracted with ethanol of various concentrations from stubbles of orchard grass after cutting on October 4

Fig. 1 shows the compositions of fractosans in the same samples shown in Table 1. The proportion of long-chain fructosans decreased during a period from Oct. 4 to Oct. 13, then it turned to increase again. Finally the proportion of long-chain fructosans on Dec. 23 became higher than that on Oct. 4. Thus, the DP of fructosans during this period seems to vary with the changes of fructosan concentration in tissues. In addition, there was another stage promoting the reaction of fructose polymerization towards the winter. The stubbles showed a similar concentration of fructosan between Nov. 19 and Dec. 23, whereas the DP value of fructosan on Dec. 23 was higher than that on Nov. 19. This additional advance of polymerization seems to be attributed to a drop of temperature to below 0°C. The quantitative and qualitative variations of fructosans described above occurred in the long-chain fructosan fractions extracted at the ethanol concentrations from 65% to 0% (water). According to estimation of the molecular weight of fructosans by the use of Sephadex G-75, the DP of fructose in these fructosans were about between 31 and 83. Namely, degradation and polymerization of carbohydrate reserves in orchard grass are mainly attributed to the alternation of amounts of long-chain fructosans: polymers consist of 31–83 fructose molecules.

Variation of non-structural carbohydrate from winter to early spring[8]

Table 2 shows the quantitative changes of carbohydrate reserves of 1- and 3-year-old orchard grasses. Both stubbles and roots on Feb. 27 contained a high level of NSC. The concentration of the total NSC hardly changed in a period from Feb. 27 to Mar. 24 in each tissue, but it steeply declined to a low level on Apr. 10. The NSC concentration in stubbles in late April became approximately one-eighth of that in late February in the 1-year-old grass and one-sixth of that in the 3-year-old grass. The extent of variation was much larger in stubbles than in roots, and larger in 1-year-old grass than in 3-year-old grass. The decrease of NSC was mainly due to the disappearance of fructosans, while total free sugar percentage changed a little during the winter. Such a decrease of NSC in the early spring began much faster in 1-year-old grass than in 3-year-old grass. As a typical example, the NSC composition of stubbles in 1-year-old grass is shown in Table 3.

Table 2. Variation of sugar and fructosan concentrations* in stubbles and roots of 1-year-old orchard grass

	Sampling date	1-year-old grass			3-year-old grass		
		Total sugar	Fructosan	Total NSC	Total sugar	Fructosan	Total NSC
Leaf sheath	Feb. 27	3.15%	28.60%	31.75%	3.48%	31.70%	35.18%
	Mar. 24	3.56	24.50	28.06	2.88	33.00	35.88
	Apr. 10	3.80	12.50	16.30	3.40	20.00	23.40
	Apr. 28	2.31	1.94	4.25	2.88	3.00	5.88
Root	Feb. 27	3.58	23.11	26.69	4.03	25.31	29.34
	Mar. 24	3.57	19.13	22.70	3.99	24.69	28.68
	Apr. 10	2.81	12.50	15.31	3.40	14.75	18.15
	Apr. 28	1.89	6.31	8.20	3.13	9.38	12.51

* % of dry weight

Table 3. NSC extracted with ethanol of various concentrations in stubbles of 1-year-old orchard grass

Per cent of ethanol	Date			
	Feb. 27	Mar. 24	Apr. 10	Apr. 28
	mg[a]	mg	mg	mg
95	1.34	1.91	2.71	2.30
85	3.15	3.56	4.80	2.31
65	5.75	6.00	6.93	3.78
50	24.50	22.31	17.00	4.25
30	31.06	28.00	17.06	4.25
Water	31.75	28.06	17.30	4.25

a) Fructose mg per 100 mg dry matter

On Feb. 27 and Mar. 24, showing only a small difference in NSC concentration between both dates, the composition of soluble carbohydrates was found to be approximately similar to each other, i.e., approximately 20% of total NSC was extracted with 65% ethanol, and 80% of that was extracted with 50% ethanol. On the other hand, on Apr. 10 the percentage of carbohydrate extracted with ethanol of lower than 65% and water was significantly decreased. Conversely, percentage of extraction increased up to 40% of total NSC with 65% ethanol, and to 98% of that with 50% ethanol. On Apr. 28, the long-chain fructosans extracted with ethanol of lower than 65% and water were scarcely found in stubbles. Such degradation of long-chain fructosan in the spring occurred not only in stubbles but also in roots. It occurred earlier in stubbles than in roots, and earlier in 1-year-old grass than in 3-year-old grass. That is to say, the new growth of grasses in early spring is initiated by the degradation and use of long-chain fructosan contained in their stubbles and roots.

Influence of fertilizer on carbohydrate reserves[9]

Influence of a particular fertilizer on the amount of carbohydrate reserves is complex because it is impossible to eliminate interferences by other nutrients and environmental factors. It has been generally known, however, that the heavy application of N decreases carbohydrate reserves while the application at low rate increases carbohydrate reserves in grasses. But, effects of other fertilizers are not so much known. Especially, the influence of fertilizer on variation of NSC composition is known little.

Table 4 and Fig. 2 show seasonal variations of NSC in leaf sheath of orchard grass grown at different levels of fertilization. Treatments were as follows. The control plot received 80 kg N, 150 kg P_2O_5 and 70 kg K_2O/ha. N, P_2O_5 or K_2O was eliminated from the above set of fertilizers for –N, –P, and –K treatments, respectively. On the contrary, N, P or K in the above set of fertilizers was replaced by 240 kg N, 450 kg P_2O_5 or 210 kg K_2O/ha

Table 4. Seasonal variation of NSC concentration* in stubbles of orchard grass as influenced by different levels of fertilizer application

Treatment	Date					
	Sept. 17	Oct. 2	Oct. 9	Oct. 16	Oct. 24	Nov. 26
Control	6.18%	28.50%	25.63%	20.63%	33.88%	48.13%
−N	25.25	37.25	25.00	28.70	38.10	46.88
−P	12.25	37.13	25.63	25.90	36.95	49.07
−K	19.60	35.63	36.25	26.90	32.45	48.75
3N	12.30	22.13	22.00	12.85	27.75	44.38
3P	6.85	37.13	30.25	21.22	32.80	46.88
3K	17.65	26.88	30.00	23.50	30.00	48.75

* % of dry weight

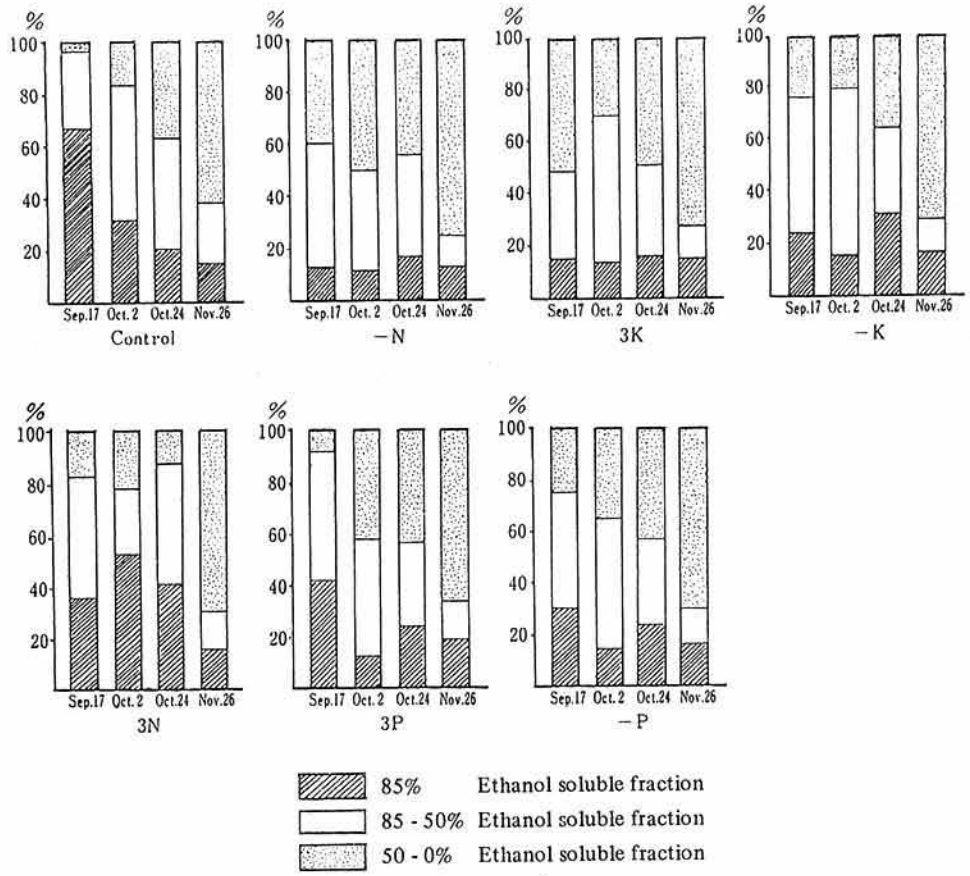

Fig. 2. Relative proportion of NSC extracted with ethanol of various concentrations from leaf sheath of orchard grass

for 3N, 3P or 3K treatment, respectively.

Plants grown in all the plots had accumulated NSC to high concentrations by Nov. 26 and their NSC was composed of highly polymerized carbohydrates. But the pattern of the accumulation was different among treatments. The –N treatment showed the highest concentration of NSC among the all plots in early autumn (25% on Sept. 17), with a high rate of long-chain fructosan (extracted at ethanol concentrations of 50–0%). The NSC concentration of 3K plot on Sept. 17 was not as high as that of the –N plot, but the DP of NSC of the former was more advanced than the latter. Compared with other treatments, the accumulation and polymerization of NSC in the 3N treatment were slow. The –K treatment showed a relatively high concentration of NSC in the early autumn (17% on Sept. 17), but the progress of polymerization was similar to 3N treatment. The concentration of NSC of both –P and 3P treatments was similar to that of the control and the polymerization proceeded steadily during the autumn. As these results show, the application of a high rate of N fertilizer delays the accumulation of NSC and the synthesis of highly polymerized carbohydrate in storage tissues of orchard grass. On the other hand, the heavy application of K fertilizer accelerates the formation of highly polymerized carbohydrates. These results suggest that the participation of K to the reaction of fructose polymerization may be analogous with starch synthesis.

Synthesis of non-structural carbohydrate from assimilated $^{14}CO_2$ in orchard grass

Distribution and incorporation of assimilated ^{14}C into NSC in plants were investigated in autumn. The top of orchard grass plants was exposed to $^{14}CO_2$ for 1 hr. The leaf blades, leaf sheaths and roots were separately harvested at 0, 1, 3, 24, 72 and 264 hr after the exposure to $^{14}CO_2$. About 30% of the fixed ^{14}C disappeared from the whole plant within 24 hr after the removal of $^{14}CO_2$, after that, the loss of radioactivity was little until 264 hr. As to the distribution of ^{14}C assimilates in plants, it was found that the leaf blades retained over 90% of the total radioactivity of the plant just after the $^{14}CO_2$ exposure, but 24 hr later it fell to 45%. The leaf sheath showed the second highest radioactivity at the initial stage and the variation of radioactivity was the smallest among plant organs until 264 hr after the exposure. On the other hand, the radioactivity of roots increased progressively with time, and it became a major sink of ^{14}C-photosynthates at 72 and 264 hr after the exposure.

Then, the incorporation of the assimilated ^{14}C into NSC was successively examined in each plant organs. With the elapse of time, the ^{14}C activity was distributed into larger carbohydrate molecules, such as short-chain fructosans in the leaf blades and long-chain fructosans in both the leaf sheaths and roots. Table 5 shows an example of time-course

Table 5. Distribution of ^{14}C activity among soluble compounds extracted with ethanol of various concentrations from leaf sheath

Time	Fraction				
	85% ethanol sol.	65% ethanol sol.	30% ethanol sol.	0% ethanol sol.(water)	Residue
0 hr	92.5%	5.1%	0.8%	0.5%	1.2%
1	92.8	3.0	1.2	0.9	3.0
3	88.7	1.5	2.5	2.5	4.8
24	57.7	5.4	15.3	13.0	8.6
72	32.8	5.0	20.4	22.3	19.5
264	14.2	5.9	21.6	27.6	30.6

changes of ^{14}C activity of extracts from leaf sheath with ethanol solutions of four grades of concentration. At 0 and 1 hr after the exposure, the most of ^{14}C activity (93%) was found in a 85% ethanol soluble fraction, followed by a 65% ethanol soluble fraction. At 3 hr after the exposure, the activity of both 30% ethanol and water soluble fractions became higher than that of the 65% ethanol soluble fraction. After that, high levels of

the activity proceeded into the NSC extracted with both 30% ethanol and water. That is to say, the assimilated ^{14}C was incorporated into long-chain fructosans with the elapse of time. However, ^{14}C activity was slightly detected in both 30% ethanol and water soluble fractions, just after the $^{14}CO_2$ exposure, and the activity in these fractions increased rapidly after that. This result supports the hypothesis[2] that the long-chain fructosans are directly synthesized by linking assimilated ^{14}C-mono-saccharides to short- or medium-chain fructosans which have been previously produced and exist in plant tissues.

References

1) DeCugnac, A.: Recherches sur les glucides des graminees. *Ann. Sci. Nat. Bot.*, 13, 1–129 (1931).
2) Kuhbauch, W. & Soberalske, R. M.: Molecular weight and ^{14}C distribution of fructosan in timothy stem bases at three stages of development. *Crop. Sci.*, 17, 239–242 (1977).
3) Ojima, K. & Isawa, T.: The variation of carbohydrate in various species of grasses and legumes. *Can. J. Bot.*, 46, 1507–1511 (1969).
4) Okajima, H. & Smith, D.: Available carbohydrate fractions in the stem bases and seed of timothy, smooth bromegrass, and several other grasses. *Crop Sci.*, 4, 317–320 (1964).
5) Percival, E. G. V.: The carbohydrate constituents of herbage. *British J. Nutr.*, 6, 104–152 (1952).
6) Smith, D.: The non-structural carbohydrates. *In* Chemistry and biochemistry of herbage 1. ed. Butler, G. W. & Bailer, R. W., Academic Press, London & New York, 105–155 (1973).
7) Sugawara, K., Isawa, T. & Hayashi, K.: Physiological studies on available carbohydrates in grasses. I. Change in sugar and fructosan composition of orchard grass with cutting. *J. Jpn. Grassl. Sci.*, 18, 202–208 (1972) [In Japanese with English summary].
8) Sugawara, K. & Isawa, T.: Physiological studies on available carbohydrates in grasses. II. Available carbohydrate of orchard grass during winter and early spring. *J. Jpn. Grassl. Sci.*, 20, 199–204 (1974) [In Japanese with English summary].
9) Sugawara, K. & Isawa, T.: Physiological studies on carbohydrates in grasses. IV. The influences of fertilizer on concentration and polymerization of non-structural carbohydrates in orchard grass. *J. Jpn. Grassl. Sci.*, 27, 352–356 (1982) [In Japanese with English summary].
10) Sugawara, K. & Isawa, T.: Physiological studies on carbohydrates in grasses. III. The fate of assimilated $^{14}CO_2$ in orchard grass leaves at fall season. *J. Jpn. Grassl. Sci.*, 24, 222–226 (1978) [In Japanese with English summary].
11) Waite, R. & Boyd, J.: The water-soluble carbohydrates of grasses. I. Changes occurring during the normal life-cycle. *J. Sci. Food Agr.*, 4, 197–204 (1953).

(Received for publication, August 2, 1982)