Effects of Surfactant Tween 80 on Enzymatic Accessibility and Degradation of Orchardgrass (*Dactylis glomerata* L.) at Different Growth Stages

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ABSTRACT : The study evaluates the enzymatic dry matter (DM) degradability and water holding capacity of leaf and stem fractions of orchardgrass (*Dactylis glomerata* L.) at different growth stages with or without the presence of surfactant Tween 80. While Tween 80 significantly ($p<0.05$) increased water and enzyme holding capacities in the leaf blades fraction, less was observed in the fraction of leaf sheath and stem of orchardgrass. The enzyme holding capacity in the leaves was also altered more than that for water holding capacity. This resulted in the increased rate and extent of enzymatic hydrolysis of the leaf blade fractions at two growth stages, whereas little was with leaf sheath and stem fractions. It was also observed that at 0.005% concentrations of Tween 80 the enzymatic DM degradability of young leaf blades was higher ($p<0.05$) by 20-30% compared to that of the control, as well as for water and enzyme holding capacity. For matured leaf blades the DM degradability were increased with over 0.01% concentrations of the surfactant, but the increase was less than leaf blades of young orchardgrass. This result suggests the possibility of using the surfactant Tween 80 to improve forage digestibility in the rumen. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 1 : 83-87)***

Key Words : Tween 80, Enzymatic Degradability, Water and Enzyme Holding Capacities

INTRODUCTION

Fibers, especially cellular membranes constitute barriers between cell compartments thereby limiting cellulolytic enzymatic degradation in forages. Although previously, quantitative studies have been focused on the need to improve forage utilization by increasing the degradability of fibrous materials using many chemical treatments (Lindberge et al., 1984; Vadiveloo, 2000). Unfortunately some detergents disrupt lipid layers as well as hydrophobic interactions that contribute to the stability of many globular proteins (Castanon and Wilke, 1981). Previous studies indicate that, the most existing difficulties in the use of commercial surfactant laid on (1) its slow hydrolysis (2) the mechanism of the hydrolysis have not been sufficiently understood and (3) its high cost of enzyme production (Castanon and Wilke, 1981; Ooshima et al., 1986; Helle et al., 1993). Therefore the need to survey a varieties of detergent with respect to their ability to solubilize cellulolytic layers without denaturing forages structural compartments of protein and lipid with respect to their effect on enzymes is imperative (Akesenova et al., 1994; Castanon and Wilke, 1981). However, little information is available on the effect of Tween 80 especially on water holding capacity and enzymatic DM degradability in young feed crops. This study evaluates effects of Tween 80 on improve enzymatic accessibility and *in vitro* DM degradability of orchardgrass (*Dactylis glomerata* L.) at two different growth stages.

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MATERIALS AND METHODS

Preparation of cell walls of the botanical fractions of orchardgrass

Orchardgrass (*Dactylis glomerata* L.) was grown in the Experimental Farm of Tohoku University (Japan) and harvested at pre-heading and middle flowering stages. The grass was manually separated to the botanical fractions and kept at -80°C until needed. Triplicate of 30 g of the frozen material was macerated in blender with 200 ml ice water three times for 30 seconds and filtered through a 300 μm nylon mesh. The residues from the filtrate (fibrous materials) were thoroughly washed with water and freeze dried. The water and enzyme holding capacities and width of the particles of the freeze dried samples were measured, and the samples were cut into waste thread by scissors, for measurements of chemical composition and dry matter (DM) degradability by a commercial cellulolytic enzyme.

Determinations of enzymatic degradation of cell wall preparations of orchardgrass

Two experiments of the *in vitro* DM degradability of cell wall preparations of orchardgrass were done using a commercial cellulolytic enzyme; the material (ca. 100 mg) was incubated at 30°C with a 30 ml of 1% solution of a commercial enzyme using a 50 ml centrifuge tube. In the first experiment with treatment of the presence or absence

of a 0.2% concentration of Tween 80, the DM degradability of leaf blades at a young growth stage and leaf blades, leaf sheaths and stems at a matured growth stage were measured after 6 h and 24 h incubation. The DM enzymatic degradability of leaf blades taken at the two growth stages were measured after 6h incubation the same as described above, under five treatments of the concentrations of Tween 80 (0.005%, 0.01%, 0.02%, 0.2% and none).

Determinations of particle size and water and enzyme holding capacity

The width of the particles was determined by spreading about 1 g of each of the three replicate on paper put on a glue-sprayed side of slide glass after they were macerated and freeze dried. The width of the particles was measured using light microscopic observation with ocular micrometer.

Water and enzyme holding capacity of orchardgrass were examined with all samples as tested for the DM degradability. The values were expressed as the amount of water and amino acids retained, respectively, after soaking it into a 1% solution of a cellulolytic enzyme used in this study for 10 min at 37°C. The soaked sample was transferred into a plastic container, which has many pinholes on the bottom after a circle filter paper was incited on the bottom to stop escape of plant material. This was carefully put into a 15 ml centrifugation tube and centrifuged at 2,000 rpm for 20 min. The container plus the material was quickly weighed and dried to determine the amount of moisture retained.

The dried samples were then used to determine the enzyme proteins attached to the fibrous particles of orchardgrass. Concentrations of aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), threonine (Thr), and tyrosine (Tyr) were used for calculating a total amount of amino acids (g kg^{-1} DM), as those of the plant and enzyme materials were more highly different than the other amino acids detected. The amino acids attached to the plant substrates and from the cellulolytic enzyme after incubation are shown in Table 1.

Determination of cell wall components and amino acids

The crude protein (CP) was determined as described by procedure 954.01 (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to methods of Van Soest et al. (1991) without the use of sodium sulfite and amylase. Acid detergent lignin (ADL) was determined using 72% H_2SO_4 solution as modified by Van Soest et al. (1991). Concentrations of amino acids of the hydrolysates recovered from a treatment with 6 N HCl at 110°C for 17 h under N_2 was measured using an amino acid analyzer (DP-8020, Tosoh Co. Tokyo Japan) (Spackman et al., 1958). The analyzer was equipped with an ion exchange column (PSK-GEL Aminopack, 4.6 mm ID×12 cm, Tosh Co. Tokyo, Japan).

Statistical analysis

Data was analyzed using an analysis of variance, and means were separated by the Turkey-Kramer test with the F-test significant at the 0.05 probability level (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Chemical composition and physical resistance of macerated samples

All cell wall preparations of orchardgrass had higher contents of cell wall constituents, irrespective of botanical fraction or harvest time, since those were obtained after

Table 1. Amino acid composition of fibrous material of orchardgrass, cellulolytic enzyme and surfactant Tween 80

Amino acids	Young orchardgrass	Matured orchardgrass			Cellulolytic enzyme	Tween 80
	Leaf blade	Leaf blade	Leaf sheath	Stem	(1:5 dilution)	(1:500 dillution)
Amino acids[1]	g kg^{-1} DM				g L^{-1}	
Aspatic	3.54	2.96	1.20	1.18	10.21	nd[2]
Glutamic	3.97	3.29	1.24	1.32	7.92	nd
Serine	1.74	1.34	0.56	0.57	6.70	nd
Histidine	0.59	0.42	0.15	0.16	0.98	nd
Glycine	1.93	1.62	0.65	0.64	5.65	0.01
Threonine	1.72	1.44	0.57	0.58	7.61	nd
Arginine	1.62	1.23	0.45	0.45	2.12	nd
Alanine	2.53	2.05	0.80	0.79	4.58	nd
Tyrosine	1.20	0.93	0.35	0.34	5.49	nd
Methionine	0.06	0.04	nd	nd	0.62	nd
Valine	2.22	1.81	0.70	0.71	3.80	nd
Phenylalanine	1.82	1.59	0.63	0.59	3.80	nd
Isolucine	1.61	1.35	0.51	0.51	2.38	nd
Leucine	3.12	2.60	1.00	1.00	5.02	nd
Lysine	2.02	1.58	0.63	0.63	2.47	nd

[1] Amino acid composition of plant, enzyme and tween 80 was determined in triplicate.
[2] nd means 'not detected'.

treatments of maceration and collection by a large mesh size. This procedure is useful to exclude soluble substances and epiderimal and mesophyll cells in leaves of perennial ryegrass (Gordon et al., 1985), and probably parenchyma cells in stems of orchardgrass as well.

The particle size of leaf blade and sheath fractions of orchardgrass was significantly ($p<0.05$) smaller than that of the stem fraction, as shown in the width of macerated samples measured by light microscopy (Table 2). The leaf blade fractions of the two growth stages were similar in contents of NDF, ADF and ADL, the averaged values of celllulose and hemicellulose fractions being calculated as 436.7 and 335.3 g kg^{-1}DM, respectively. Within same harvest time of middle flowering stage, leaf blades and leaf sheaths had lower NDF and ADF contents than the stem fraction, and a slightly higher ADL content. Thus, large differences of cellulose content between the three botanical fractions were observed.

The fact is consistent with a gravitrical analysis of forage maize, which showed that the major component of the culm is fibrous tissue walls such as sclerenchyma and vascular vessel walls (Wilson, 1993). It was also reported from anatomical study that the parenchyma tissue walls of the culm fraction of forage maize had lower cell wall constituents than the whole other tissue walls. Electron microscopic observation of forage digestion by rumen microorganisms and/or their associated enzymes has shown that the mesophyll and parenchymal cell walls are much more degradable than the other tissue walls (Goto et al., 1993).

Differences of effects of Tween 80 among three botanical fractions and two growth stages on the enzyme accessibility and degradability of orchardgrass

The water holding capacity of leaf blades in both young and matured orchardgrass significantly ($p<0.05$) increased in the presence of the surfactant Tween 80, whereas little effect was observed for the leaf sheath and stem fractions in matured orchardgrass (Table 3). Compared between two growth stages, the increase in water holding capacity of the leaf blade fraction was also higher with young plant than the mature one. The amount of water retention in leaf blade fractions at the two growth stages and leaf sheath fraction was almost the same, but lower than that of the stem fraction.

The significantly higher enzyme holding capacity of the leaf blade fraction in both young and matured orchardgrass was observed in the presence of the surfactant Tween 80 ($p<0.05$), and was not with leaf sheath and stem fractions in matured orchardgrass. However, the greater increase of enzyme holding capacity compared to that of water holding capacity was observed with the leaf blade fraction, as shown by the increment rate. The fact could be related to differences in the binding mode and strength of the enzyme and water against the centrifugation force. Thus, the enzyme accessibility of leaf blade fractions was increased by the presence of Tween 80.

For both young and matured plants, the enzymatic degradability of the leaf blade was significantly ($p<0.05$) higher with the presence of Tween 80 than without that of the surfactant (Table 4). Within young leaf blades

Table 2. Chemical composition and width of fibrous material of the botanical fractions of orchardgrass at different growth stages

	Grass composition (gkg^{-1} DM)						Width of particle (mm)
	Crude protein	NDF	ADF	ADL	Cellulose	Hemi-cellulose	
Young orchardgras							
Leaf blade	29.9	830.3	500.8	66.7	434.1	329.5	0.11 ± 0.05^{c}
Matured orchardgrass							
Leaf blade	25.4	838.2	497.1	57.9	439.2	341.1	0.15 ± 0.08^{c}
Leaf sheath	7.4	903.0	544.0	38.0	506.0	359.0	0.28 ± 0.12^{b}
Stem	5.3	923.4	589.0	24.7	564.3	334.4	0.66 ± 0.21^{a}

Means with different superscripts within the column differed at ($p<0.05$).
NDF=Neutral detergent fiber, ADF=acid detergent fiber, ADL=acid detergent lignin.

Table 3. Effect of surfactant Tween 80 on water and enzyme holding capacity of fibrous material of orchardgrass

	Young orchardgrass	Matured orchardgrass		
	Leaf blade	Leaf blade	Leaf sheath	Stem
Water holding capacity		H_2O g kg^{-1} DM		
Control	$1,840\pm200^{b}$	$1,800\pm230^{a}$	$1,770\pm110^{b}$	$2,310\pm90^{a}$
Tween 80	$2,260\pm60^{a}$	$2,040\pm60^{a}$	$2,040\pm60^{a}$	$2,130\pm20^{b}$
Enzyme holding capacity		Amino acids g kg^{-1} DM		
Control	13.1 ± 3.6^{b}	39.6 ± 6.9^{b}	32.4 ± 2.9	36.5 ± 7.4
Tween 80	90.1 ± 6.7^{a}	59.8 ± 5.6^{a}	30.1 ± 2.7	32.3 ± 32.3

Means with different superscripts within the same column differed at ($p<0.05$).
Each value represents means of five replicate.

Table 4. Effects of surfactant Tween 80 on orchardgrass degradability (g kg^{-1} DM) by cellulolytic commercial enzyme

	After 6 h Incubation		After 24 h Incubation	
	Control	Tween 80	Control	Tween 80
Young orchardgras				
Leaf blade	346.8±48.5^{b}	468.8±20.1^{a}	558.8±7.5^{b}	581.7±3.4^{a}
Matured orchardgrass				
Leaf blade	287.8±42.7	330.7±38.4	439.7±2.6^{b}	473.6±3.6^{a}
Leaf sheath	343.8±10.1^{b}	360.9±6.0^{a}	300.3±12.8	299.2±8.8
Stem	251.9±18.5	261.3±36.4	345.7±6.0	347.4±5.7

Means with different superscripts within the same column differed at ($p<0.05$).
Each value represents mean of five replicate.

differences in the enzymatic degradability between absence and presence of Tween 80 was more pronounced at a 6 h incubation than at a 24 h. There were little effects of Tween 80 on the DM degradability of leaf sheath and stem fractions, up to 24 h incubation.

Differences in effects of different concentrations of Tween 80 on the enzyme accessibility and degradability of orchardgrass leaves

With over 0.005% concentrations of Tween 80 the enzymatic DM degradability of young leaf blades was significantly ($p<0.05$) increased as compared to that of the control (Figure 1). The increment of those values showed

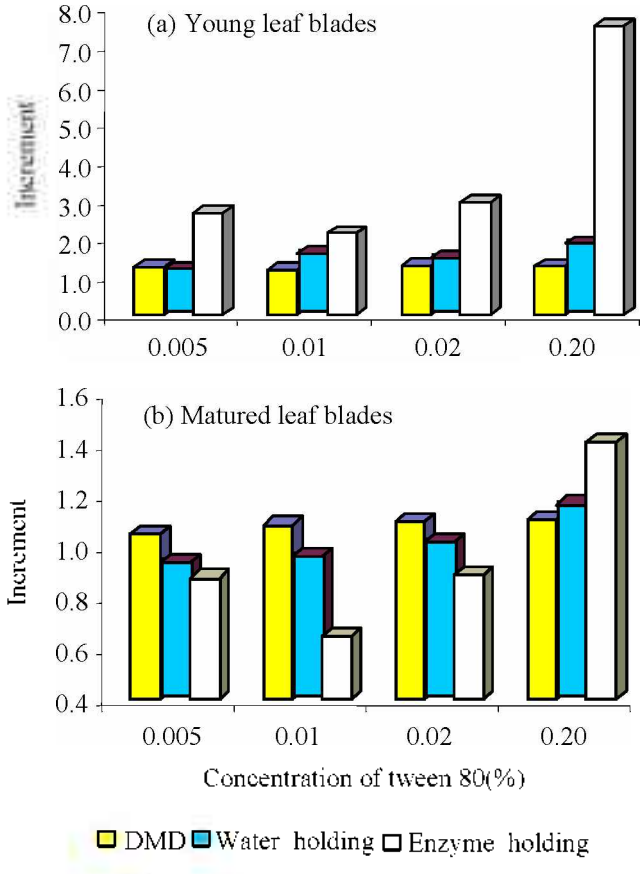

Figure 1. Effect of surfactant Tween 80 on water holding, energy holding and enzymatic degradation of botanical fraction of orchardgrass at different growth stages.

1.2-1.3 times at any concentration of Tween 80, although it tended higher at over 0.2% of the surfactant. The enzyme holding capacity of young plant significantly ($p<0.05$) increased, the increment showing 2.1 to 2.9 times and 7.5 times of the control at 0.005-0.02% and 0.2% concentrations of Tween 80, respectively.

For matured leaf blades the DM degradability were increased with over 0.01% concentrations of the surfactant, but was much less than young orchardgrass. The increase of enzyme holding capacity was observed only with a 0.2% concentration of the surfactant, and was no increases of water holding capacity throughout all Tween 80 concentrations. Thus, although the enzymatic degradation of the leaves were improved with treatment of Tween 80, the response of DM degradability to the surfactant concentration was inconsistent with the response of enzyme holding capacity to the surfactant concentration.

Thus, the improvement of DM degradability of orchardgrass by the surfactant Tween 80 is in good agreement with previous result of enzymatic degradation of filter paper (Castanon and Wilke, 1981). Since the presoaking of seeds into Tween 80 can promote the germination and strengthen the drought resistance of wheat plant (Aksenova et al., 1994), the greater enzyme holding capacity of the surfactant-exposed orchardgrass observed in this study can be related to physicochemical loosening of the structure of forage cell walls. The fact may further bring cellulolytic enzymes into quick and intimate contact with accessible and otherwise inaccessible substrate sites of orchardgrass cell walls. Otherwise, the surfactant can prolong the enzyme activity, probably by facilitating its dissolution before the catalytic activity becomes inactive (Steve et al., 1993). It is, however, in this study unknown of how the response of DM degradability to the surfactant concentration is inconsistent with the response of enzyme holding capacity to the surfactant concentration. Therefore, the improvement of the rate and extent of enzymatic degradation of orchardgrass tissues obtained by Tween 80 in this study is due to the improvement of the interaction of cellulolytic enzyme and cell wall components such as cellulose and hemicellulose.

CONCLUSION

The result of this study suggests that the presence of the surfactant Tween 80 altered the water absorption and enzyme accessibility of orchardgrass cell wall and improved its enzymatic DM degradability. More research is needed to evaluate whether the cell wall degradability of forages by rumen microorganisms can be improved either with pretreatment of Tween 80 alone or with that of commercial enzyme and Tween 80.

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