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THE EFFECTS OF CELLULASE ON CELL WALL STRUCTURE AND THE RUMEN DIGESTION OF ALFALFA SILAGE

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Abstract

First- and second-cut alfalfa (*Medicago sativa*) was ensiled with no additive, microbial (*Lactobacillus casei*) inoculant, cellulase derived from *Acremonium celluloytics* Y-94, co-addition of inoculant and cellulase, and formic acid. The resultant silages were digested in the rumen of a dairy cow. The alfalfa and the silages were then examined with scanning electron microscope (SEM) and their chemical characteristics analyzed to evaluate the effects of cellulase on the quality of alfalfa silage and its cell wall structure.

The addition of cellulase lend to both a greater loss of parenchymal tissue and decrease in digestibility during rumen degradation than did the other additives moreover, photos taken during SEM examination also showed that cellulase affected cell wall decomposition. The results of this study may suggest that the addition of cellulase affects fiber digestion by ruminant animals.

Keywords: Cellulase, silage, cell wall, scanning electron microscope

Introduction

Previous studies have reported that the addition of cellulase produces an improvement in silage quality (Aniwaru et al., 1998). The addition of cellulase also results in differences in the digestibility of fiber fractions. However, there is a paucity of information

on the effects of cellulase on cell wall structure during the silage fermentation and its digestion during rumen fermentation. The objective of this study was to evaluate the effects of the addition of cellulase on first- and second-cut alfalfa silages on change in cell wall structure during ensiling and rumen fermentation by analysis of scanning electron microscope (SEM) examination.

Material and Methods

First- and second-cut alfalfa was ensiled with either no additive (control); 0.02 g kg^{-1} of microbial (*Lactobacillus casei*) inoculant (LC); 0.1 g kg^{-1} of cellulase derived from *Acremonium celluloyticus* Y-94 (AC); 0.02 g kg^{-1} of LC and 0.1 g kg^{-1} of AC (LC+AC), or 5 g kg^{-1} of formic acid (FA), in 1-litter plastic silos for 50 d. The second fraction of the alfalfa stem was cut to 2 cm and ensiled with the above silages in 8 plastic bags per silo for the observation of cell wall structure. After the opening of the silages, 4 stem samples were settled in the rumen of a cow for 48 h through a rumen cannula. The remaining 4 samples, non-ensiled cut samples of cut alfalfa, and the samples placed in the rumen were observed by SEM.

Results

The AC treatment resulted in a greater reduction of pith parenchymal tissue in both first and second cuts than did the other treatments, and the physical decomposition of cell wall structures was observed under SEM in the samples treated with AC and LC+AC. The neutral detergent fiber (NDF) content of the first-cut silage was significantly lower in the FA-treated samples than in the other first-cut silages, and both AC and LC+AC treatments resulted in significantly lower NDF contents. The addition of formic acid resulted in a significantly lower acid detergent fiber (ADF) content in the first-cut silages, and there was a tendency for the

control silage to be lower in ADF content. All second-cut silages, except the control, were low in ADF content.

Pith parenchymal, tissues in all the samples disappeared during rumen degradation, and the physical decomposition of the cell wall was observed in LC+AC-treated of first-cut silage, and LC- and FA- treated second-cut silages. NDF degradation through the rumen digestion was significantly lower in AC- and LC+AC- treated first-cut, and in the control and LC- and AC- treated second cut silages.

Discussion

Aniwaru et al. (1997) reported that the addition of cellulase derived from *Acremonium celluloyticus* Y-94 to silage lowers the NDF content. A similar tendency was observed in the second-cut silages in this study, and NDF and ADF contents in the first-cut LC+AC-treated silages also tended to be lower. Aniwaru et al. (1997) also reported that lignifications of parenchymal tissue appeared low when examined with a video microscope, and that the parenchymal tissue of silage disappeared faster than the other tissue when cellulase was added. The physical decomposition of the cell wall and the disappearance of parenchymal tissue were also observed in this study using an SEM.

Disappearance of parenchymal tissue in all samples through rumen degradation was demonstrated both by chemical analysis and SEM examination. Some studies have pointed a tendency of higher in vitro DM digestibility (IVDMD) when cellulase is added to silage, but other studies have reported opposite tendencies; i.e., that the addition of cellulase tended to decrease IVDMD (Matsuoka et al., 1997; Zhuang et al., 1999). The chemical analysis and SEM examination in this study also indicated that the addition of cellulase lead to a decrease in the digestibility of alfalfa silage. Both video microscope examination (Okamoto et al., 1994) and the SEM examination in this study indicated the disappearance of parenchyma

tissues, which seemed to be low in lignification.

The results of this study may suggest that the addition of cellulase to alfalfa prior to ensiling degrades the more easily degradable part of the cell wall as well as the less-degradable part of the cell wall. Hence, we conclude that addition of cellulase affects fiber digestion by ruminant animals.

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Table 1 - Cell wall consituent and in situ digestibility of alfalfa silages.[1]

Cutting	Additives	Silage		Digestibility	
		NDF	ADF	NDF	DM
		gkg^{-1}DM		gkg^{-1}	
First	Control	539^{a}	455^{Aa}	223^{ABbc}	275^{b}
	LC	530^{ab}	450^{ABa}	319^{Aa}	371^{a}
	AC	541^{a}	447^{ABa}	158^{Bc}	262^{b}
	LC+AC	518^{ab}	427^{Bb}	180^{Bc}	321^{ab}
	FA	495^{b}	395^{Cc}	290^{ABab}	372^{a}
Second	Control	507^{c}	446^{c}	287^{Dd}	435^{d}
	LC	471^{d}	409^{d}	295^{Dd}	402^{cd}
	AC	452^{d}	390^{d}	258^{Dd}	413^{cd}
	LC+AC	452^{d}	388^{d}	231^{Dd}	413^{cd}
	FA	507^{cd}	406^{d}	406^{Cc}	359^{c}

[1)]Means within the same coloumn and hearvest with different superscripts differ.
($^{A,B,C,D}P<0.01$, $^{a,b,c,d}P<0.05$)