

**THE EFFECT OF CELLULASE ON CELL WALL STRUCTURE AND  
THE RUMEN DIGESTION OF TIMOTHY SILAGE**

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**Abstract**

The objective of this study was to determine the effect of additives on the structure changes of related tissues during the ensiling process and the rumen digestion of timothy. In the first cut-timothy, the addition of LC+AC improved the fermentation qualities of the silage. Addition of cellulase resulted in significant decreases in NDF, ADF, cellulose, and hemicellulose content. SEM examination of the samples suggests that the degradation of parenchymal tissues was enhanced by the cellulase, but no significant differences were observed among the additives in the rumen digestion. The NDF and cellulose digestibility of the AC- and LC+AC-treated silages were lower than those of the other silages. In the second one, after digestion in the rumen, there was a marked loss of inner parenchymal tissues in AC- and LC+AC-treated silages.

**Keywords:** Additives, timothy, silage, scanning electron microscope, tissue structure.

**Introduction**

There have been many studies on the effects of additives such as *Acremonium* cellulase and lactic acid bacteria on the degradation of nutrients and changes in plant tissue structure during the ensiling process (McDonald et al., 1991; Ataku et al., 1993; Aniwaru et al., 1997 and 1999). However, there are insufficient informations on the effect of cellulase on changes in plant tissue structures during the silage fermentation and its digestion in the rumen. The objective of this study was to determine the effect of additives on the digestibility of timothy silage and the structure changes of related tissues both in the silos and in the rumen.

### **Material and Methods**

First-and second-cut timothy was cut into 2-cm lengths, placed separately into small nylon bags with the ensiling materials, and ensiled in 1-liter experimental silos(8 bags per silo). At the ensiling, various additives were mixed with ensiling materials with a spray. The treatments included 0.02gkg<sup>-1</sup> of microbial( *Lactobacillus casei*) inoculant(LC), 0.1 gkg<sup>-1</sup> of cellulase additive derived from *Acremonium cellulolyticus*(AC), 0.02gkg<sup>-1</sup> of LC and 0.1gkg<sup>-1</sup> of AC (LC+ C), and 3gkg<sup>-1</sup> of formic acid (FA). No additives were mixed in the control silage. Each treatment was duplicated and ensiled for 50 d, after which half of the samples were frozen in their nylon bags and preserved until scanning electron microscope(SEM) examination. The other half of the samples and grass were put in the rumen of a cow for 48 h, then removed and frozen. All of the samples were examined with SEM and cell wall components were analyzed.

### **Results and Discussion**

In the first cut-timothy, the addition of LC+AC improved the fermentation qualities of the silage. Addition of AC both and LC+AC resulted in significant decreases in neutral detergent fiber(NDF), acid detergent fiber(ADF), cellulose, and hemicellulose content. SEM examination of the samples suggests that the degradation of parenchymal tissues was enhanced by the cellulase, but no significant differences were observed among the additives during the rumen digestion. The NDF and cellulose digestibility of the AC- and LC+AC-treated silages were lower than those of the other silages.

In the second cut timothy, vascular bundles in the control treatment, LC, AC and LC +AC treatments remained forms like a peninsula, but no changes in the FA treatment. After digestion in the rumen, there was a marked loss of inner parenchymal tissues in AC- and LC +AC-treated silages, but no significant changes were observed in the untreated the control silage.

## References

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**Table 1** - Chemical composition of silages<sup>1</sup>

Cut	Treatments Additive	Moisture	CP	WSC	NDF	ADF	ADL	CEL	HEM
First	Control	826 <sup>Aa</sup>	86 <sup>Bc</sup>	08 <sup>Bc</sup>	722 <sup>Aa</sup>	476 <sup>Aa</sup>	59 <sup>a</sup>	416 <sup>Aa</sup>	246 <sup>Aba</sup>
	LC	805 <sup>BCc</sup>	115 <sup>Ab</sup>	08 <sup>Bbc</sup>	711 <sup>ABa</sup>	438 <sup>Bb</sup>	54 <sup>ab</sup>	384 <sup>BCb</sup>	273 <sup>Ab</sup>
	AC	824 <sup>Aa</sup>	111 <sup>Ab</sup>	10 <sup>Bbc</sup>	676 <sup>BC</sup>	417 <sup>BCb</sup>	55 <sup>ab</sup>	363 <sup>CDc</sup>	258 <sup>Abab</sup>
	LC+AC	817 <sup>ABa</sup>	122 <sup>Aa</sup>	15 <sup>Bb</sup>	632 <sup>Cd</sup>	389 <sup>Cc</sup>	57 <sup>a</sup>	332 <sup>Dd</sup>	243 <sup>Ba</sup>
	FA	802 <sup>Cb</sup>	116 <sup>Aab</sup>	31 <sup>Aa</sup>	694 <sup>ABb</sup>	425 <sup>Bb</sup>	45 <sup>b</sup>	380 <sup>BCb</sup>	269 <sup>Abb</sup>
Second	Control	758	114	18 <sup>Cb</sup>	612 <sup>AB</sup>	367 <sup>ab</sup>	55	312 <sup>a</sup>	244 <sup>B</sup>
	LC	753	132	30 <sup>ABa</sup>	631 <sup>A</sup>	369 <sup>a</sup>	59	309 <sup>ab</sup>	263 <sup>A</sup>
	AC	757	136	26 <sup>AB</sup>	550 <sup>B</sup>	340 <sup>b</sup>	58	281 <sup>c</sup>	210 <sup>Cb</sup>
	LC+AC	762	124	26 <sup>AB</sup>	569 <sup>B</sup>	345 <sup>ab</sup>	57	288 <sup>b</sup>	224 <sup>Ca</sup>
	FA	749	128	48 <sup>A</sup>	631 <sup>AB</sup>	369 <sup>a</sup>	59	309 <sup>ab</sup>	261 <sup>A</sup>

<sup>1</sup>CP – crude protein; WSC – water soluble carbohydrate; NDF – neutral detergent fiber; ADF – acid detergent fiber;

Adl – acid detergent lignin; CEL – cellulose (ADF-ADL); HEM – hemicellulose (NDF-ADF).

A,B,C,D : p<0.01; a,b,c,d : p<0.05.

**Table 2** - Chemical composition of silages<sup>1</sup> after ruminal fermentation

Cut	Treatments	CP	NDF	ADF	ADL	CEL	HEM
		(gkg <sup>-1</sup> DM)					
First	Control	125	774	468	105	337	332 <sup>ABb</sup>
	LC	135	770	437	106	324	340 <sup>ABab</sup>
	AC	124	771	445	116	309	346 <sup>ABa</sup>
	LC+AC	136	763	442	103	330	330 <sup>Bb</sup>
	FA	131	783	450	100	331	353 <sup>Aa</sup>
Second	Control	144	750	424	81	343	326
	LC	130	780	437	94	343	343
	AC	143	783	419	88	331	364
	LC+AC	145	755	429	96	334	326
	FA	149	766	419	87	332	347

<sup>1</sup>CP – crude protein; WSC – water soluble carbohydrate; NDF – neutral detergent fiber; ADF – acid detergent fiber;

Adl – acid detergent lignin; CEL – cellulose (ADF-ADL); HEM – hemicellulose (NDF-ADF).

B : p<0.01; a b : p<0.05.