INDIGENOUS MICROFLORA ON ALFALFA AND CORN, AND POPULATION CHANGES DURING ENSILING[1,2]

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Summary

Lactic acid bacteria (LAB), Enterobacteriaceae, yeasts, molds, and lactate-using yeasts were examined on four cuttings of alfalfa, each at three maturity stages, and three corn hybrids in 1989. In addition, microflora population changes were traced during ensiling for the second and fourth cutting alfalfas and the three corn hybrids.

Enterobacteriaceae were predominant on alfalfa; yeasts, molds, and Enterobacteriaceae predominated on corn. Higher proportions of lactate-using yeast were found on corn than alfalfa. Lactic acid bacteria comprised a small (10^4 to 10^5 CFU/g) proportion of the total (10^6) populations, with streptococci the main indigenous LAB group. Lactobacilli, pediococci, and leuconostoc were the minor groups, and their occurrence was variable, particularly on alfalfa. Cutting and maturity of alfalfa did not have a significant effect on the indigenous microflora. The chopping process significantly increased the numbers of microorganisms, but wilting alfalfa did not affect the populations.

Once the crops were ensiled, LAB grew extremely fast, and reached maximum numbers at 3 d post-ensiling. Yeast and mold counts showed a continuous reduction as ensiling progressed, and this was much more pronounced in alfalfa than corn.

(Key Words: Alfalfa, Corn, Microflora, Silage.)

Introduction

The indigenous microflora present on forages is responsible for silage fermentation, unless a commercial inoculant is added. These indigenous (or epiphytic) microorganisms are naturally occurring on all crops and mainly comprise bacteria and fungi. Lactic acid bacteria (LAB) ferment carbohydrates to lactic acid, which lowers the pH of the ensiled crop. Enterobacteriaceae, yeasts, and molds are naturally present on most crops, and their existence in silage is usually considered detrimental. Enterobacteriaceae, called acetic acid bacteria, ferment carbohydrates to acetic acid, resulting in a slowing-down of the silage pH drop. Yeasts and molds not only compete with LAB for carbohydrates, but ferment lactic acid, leading to

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increased pH and increased dry matter loss. Yeasts, particularly lactate-using yeasts, also contribute to the aerobic instability of silage.

Only limited information is available concerning the indigenous microflora on the silage crops grown in Kansas (see page 114 of this report). Our purposes were to qualitate and quantitate the indigenous microorganisms present on alfalfa and whole-plant corn in 1989, and to trace the development (i.e., changes) of the microbial population during the ensiling process.

Experimental Procedures

A second-year stand of Cody alfalfa was examined in 1989 at second, third, fourth, and fifth cuttings and at the late-bud, 10% bloom, and 50% bloom maturity stages within each cutting. Three Pioneer corn hybrids (3389, 3377, and 3379) were grown under irrigation and harvested at two-thirds milk line kernel maturity.

Three alfalfa samples (standing crop, windrow prior to chopping, and post-chopping) and two corn samples (standing crop and post-chopping) were taken from the center of each field. Alfalfa was wilted in the swath for 4 to 6 hr prior to chopping. Chopped crops were ensiled in PVC laboratory silos by procedures described on page 105 of this report. Three silos were opened at 1, 3, 7, 42, and 90 d post-ensiling. Six and 12 hr openings were added to the corn silage sampling schedule.

Microbial evaluations. Standing crop and windrow samples were cut with scissors prior to making the dilutions. Fifty grams of each sample was weighed into a blender jar containing 450 ml sterile buffer and homogenized for 40 sec. Tenfold dilutions were then made with sterile buffer, and the following microorganisms were counted:

Lactic acid bacteria. Rogosa SL medium (Difco) was used for lactobacilli, pediococci, and leuconostoc counts. Plates were overlaid with the same medium and incubated at 37 C for 2 d. Streptococci were enumerated in Slanetz & Bartley medium (Oxiod) after incubation at 37 C for 2 d.

Enterobacteriaceae. Violet red bile agar (Difco) plus 1% glucose was employed, using a pour plate technique. Incubations were at 37 C for 2 d.

Yeasts and molds. Malt agar (Difco) was used, with penicillin and streptomycin (60 μg/ml) to kill bacteria. Counts were made following incubation at 21 C for 2 d.

Lactate-using yeasts. Yeast nitrogen base agar (Difco) was used, with lactic acid as the sole source of energy. The plates were incubated at 30 C for 3 d.

The 12 alfalfa silages examined here were also used in the study found on page 102 of this report.

Results and Discussion

Presented in Table 40.1 are the indigenous microbial profiles on the 12 harvests of alfalfa and three hybrids of corn. Enterobacteriaceae were predominant on both alfalfa and corn. Lactic acid bacteria comprised a small proportion of the total microbial populations on

Table 40.1. Indigenous Microflora on Alfalfa and Corn

Crop	Cutting or hybrid	Maturity	Lactobacilli, pediococci, and leuconostoc	Strepto-cocci	Total lactic acid bacteria[1]	Entero-bacter-iaceae	Yeasts and molds	Lactate-using yeasts
			---------- log_{10} colony-forming units/g of crop[2] ----------					
Alfalfa	2	Late-bud	1.00	1.30	1.48	5.93	4.32	—
		10% bloom	2.65	3.30	3.39	6.05	5.58	4.59
		50% bloom	3.53	3.88	4.04	5.82	5.40	4.80
	3	Late-bud	1.30	2.04	2.11	5.02	3.70	3.86
		10% bloom	4.70	4.66	4.98	5.74	4.36	3.55
		50% bloom	3.38	4.76	4.78	6.03	5.28	4.80
	4	Late-bud	3.32	3.66	3.83	5.73	5.14	3.46
		10% bloom	2.23	3.72	3.73	5.44	5.10	3.56
		50% bloom	4.05	5.07	5.11	5.69	5.26	3.56
	5	Late-bud	1.30	3.07	3.08	6.83	4.82	2.79
		10% bloom	<1.00	2.48	2.49	6.11	3.70	2.73
		50% bloom	<1.00	2.18	2.20	3.00	3.70	2.32
	Average		3.76	4.31	4.41	6.06	5.08	4.23
Corn	3389		3.08	2.88	3.29	5.32	5.28	3.54
	3377		4.68	4.72	5.00	6.16	6.67	6.50
	3379		3.17	5.35	5.35	6.89	7.24	6.56
	Average		4.23	4.96	5.04	6.50	6.85	6.36

[1]Sum of lactobacilli, pediococci, leuconostocs, and streptococci.
[2]Log_{10} of 1 = 10, log_{10} of 3 = 1,000, log_{10} of 6 = 1,000,000.

alfalfa, and streptococci were the main lactic acid bacteria. Lactobacilli, pediococci, and leuconostoc counts were small and extremely variable, particularly among the alfalfas. The higher numbers of indigenous LAB on corn compared to alfalfa were principally due to streptococci. All three corn hybrids showed relatively high yeast and mold populations.

Neither cutting nor maturity of alfalfa significantly influenced the indigenous microflora ($P>.05$; Table 40.2). The third and fourth cuttings did have numerically (but not significantly) higher populations of LAB than the second and fifth.

The chopping process significantly increased LAB and Enterobacteriaceae ($P<.05$) populations (Table 40.3) for both alfalfa and corn. However, the indigenous microflora of alfalfa was not affected by wilting in the field prior to chopping.

As expected, dramatic changes in the microflora occurred during the ensiling process (Table 40.4). The LAB quickly proliferated, and lactobacilli, pediococci, and leuconostoc grew as rapidly as streptococci in the early stages. The LAB population was maximum (10^9/g) at 3

Table 40.2. Effects of Cutting and Maturity on Indigenous Microflora on Alfalfa

Item	Lactobacilli, pediococci, and leuconostoc	Strepto-cocci	Total lactic acid bacteria[1]	Entero-bacteri-aceae	Yeasts and molds	Lactate-using yeasts
	---------- log_{10} colony-forming units/g of crop[2] ----------					
Cutting						
2	3.11	3.51	3.65	5.95	5.34	4.71[a]
3	4.24	4.54	4.72	5.76	4.86	4.39[ab]
4	3.65	4.63	4.67	5.66	5.18	3.53[b]
5	1.78	2.73	2.78	6.43	4.41	2.65[b]
Maturity						
Late-bud	2.74	3.17	3.31	6.31	4.76	3.56
10% bloom	4.10	4.13	4.42	5.92	5.13	4.06
50% bloom	3.63	4.66	4.70	5.75	5.20	4.51

[1]Sum of lactobacilli, pediococci, leuconostocs, and streptococci.
[2]Log_{10} of 1 = 10, log_{10} of 3 = 1,000, log_{10} of 6 = 1,000,000.
[ab]Values among cuttings with different superscripts differ (P<.05).

Table 40.3. Effect of Pre-treatments on Indigenous Microflora of Alfalfa and Corn

Crop	Population	Standing	Windrow	Post-chopping
		-- log_{10} colony-forming units/g of crop[2] --		
Alfalfa	Lactobacilli, pediococci, and leuconostoc	3.76[b]	3.08[b]	5.10[a]
	Streptococci	4.31[b]	4.25[b]	5.22[a]
	Total LAB[1]	4.41[b]	4.28[b]	5.46[a]
	Enterobacteriaceae	6.06[b]	6.32[ab]	6.70[a]
	Yeasts and molds	5.08	5.00	5.30
	Lactate-using yeasts	4.23	3.79	6.41
Corn	Lactobacilli, pediococci, and leuconostoc	4.23[b]	—	6.24[a]
	Streptococci	4.96	—	6.85
	Total LAB[1]	5.04[b]	—	7.00[a]
	Enterobacteriaceae	6.50[b]	—	7.49[a]
	Yeasts and molds	6.85	—	7.12
	Lactate-using yeasts	6.36	—	6.65

[1]Sum of lactobacilli, pediococci, leuconostocs, and streptococci.
[2]Log_{10} of 1 = 10, log_{10} of 3 = 1,000, log_{10} of 6 = 1,000,000.
[ab]Values in the same row with different superscripts differ (P<.05).

d post-ensiling. There were no significant differences between alfalfa and corn silages, even though the LAB counts were much higher on corn at the time of ensiling. Lactic acid bacteria numbers tended to decrease, particularly after d 7, with streptococci showing a faster decline than lactobacilli, pediococci, and leuconostoc. Yeast and mold counts tended to decrease as ensiling progressed, and this was much more pronounced with alfalfa than corn.

Numerous European studies have shown that the indigenous LAB counts on grasses and legumes are variable and much lower than our results for alfalfa and corn. Geography and/or climatic effects likely contribute to these differences. Our observation of more LAB on the standing corn than alfalfa could reflect a crop influence related to substrate, especially soluble carbohydrates. A proliferation of LAB at the early stages of ensiling is essential, if good preservation and minimum nutrient loss are to be achieved.

Other European results have indicated that Enterobacteriaceae dominate silage microflora during the first few days of ensiling and then are replaced by LAB. However, this did not occur in our studies; Enterobacteriaceae numbers declined after d 1 in both alfalfa and corn. Enterobacteriaceae and molds are sensitive to low pH, which prevents their growth, but yeasts survive a pH as low as 2.5. If yeasts proliferate during the ensiling process, the silage may be unstable when exposed to air, especially in silages rich in soluble carbohydrates and/or lactic acid.

Table 40.4. Changes in the Indigenous Microflora of Second and Fourth Cutting Alfalfa and Corn during the Ensiling Process

Silage	Population	Time of fermentation, d							
		0	1/4	1/2	1	3	7	42	90*
		---------- log_{10} colony-forming units/g of crop[2] ----------							
Alfalfa	Lactobacilli, pediococci, and leuconostoc	5.30	—	—	8.92	9.45	9.29	8.43	8.38
	Streptococci	5.30	—	—	8.97	9.17	8.93	7.63	7.05
	Total LAB[1]	5.60	—	—	9.25	9.67	9.45	8.49	8.40
	Enterobacteriaceae	6.84	—	—	7.12	6.65	6.08	2.30	0.00
	Yeasts and molds	5.90	—	—	5.49	4.37	3.76	1.08	1.30
	Lactate-using yeasts	4.53	—	—	—	—	—	1.00	1.00
Corn	Lactobacilli, pediococci, and leuconostoc	6.24	7.63	8.43	8.89	9.04	8.81	7.62	5.00
	Streptococci	6.85	7.84	8.31	8.77	8.82	8.44	5.78	3.60
	Total LAB[1]	7.00	8.05	8.67	9.13	9.24	8.97	7.62	5.00
	Enterobacteriaceae	7.49	7.48	7.05	7.32	5.15	5.16	4.56	2.30
	Yeasts and molds	7.12	7.18	6.90	6.74	5.26	5.77	6.36	4.16
	Lactate-using yeasts	6.65	—	—	—	—	—	6.33	3.58

[1]Sum of lactobacilli, pediococci, leuconostocs, and streptococci.
[2]Log_{10} of 1 = 10, log_{10} of 3 = 1,000, log_{10} of 6 = 1,000,000.
*120 d for corn silage.