Survival of *Escherichia coli* 0157:H7 Added to Grass at Ensiling and Its Influence on Silage Fermentation

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SURVIVAL OF *ESCHERICHIA COLI* 0157:H7 ADDED TO GRASS AT ENSILING AND ITS INFLUENCE ON SILAGE FERMENTATION

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Abstract

Escherichia coli can cause health problems in humans and livestock. It is frequently found in faeces and soil, both of which can contaminate grass harvested for silage-making. To determine the survival of *E. coli* 0157:H7 during ensilage, and its influence on ensilage, unwilted precision-chop grass was ensiled in laboratory silos with the following additive treatments: (a) no additive, (b) *E. coli* 0157:H7 (Ec - non-infectious strain, at $\log_{10}$ 4.5 colony forming units (cfu) g^{-1}), formic acid (FA), and (d) Ec + FA. Silos were stored at 15^{o}C. Three silos per treatment were opened on days 0, 2, 5, 9, 19 and 180 of ensilage. Silages made without additive or with formic acid underwent contrasting but rapid, lactic acid dominant fermentations. Formic acid restricted fermentation, reducing ($P<0.001$) buffering capacity and the concentration of lactic acid and increasing ($P<0.001$) the concentration of water soluble carbohydrates. Counts of indigenous Enterobacteria were initially high ($\log_{10}$ 8.1 cfu g^{-1}) but declined rapidly in the early stages of ensilage and were not detected by day 19 of ensilage. Formic acid increased ($P<0.05$) the initial rate of decline in enterobacterial numbers. No indigenous *E. coli* 0157 were found on the ensiled grass. Inoculation with *E. coli* 0157:H7 increased ($P<0.001$) the numbers of this organism but they declined rapidly and

were absent by day 5 of ensilage. The addition of formic acid accelerated ($P<0.001$) this rate of decline. The added *E. coli* did not alter ($P>0.05$) silage fermentation pattern.

Keywords: Enterobacteria, Escherichia, E. coli, grass, silage, survival, fermentation

Introduction

Enterobacteria can negatively influence silage fermentation, particularly where the initial rate of pH decline is slow. These gram negative, non-sporing, rod shaped and facultatively anaerobic bacteria ferment monosaccharides to short-chain organic acids (especially acetic acid) or butanediol. They have weak proteolytic activity but can deaminate and decarboxylate some amino acids. Most species can reduce nitrate via nitrite to ammonia, and some can also produce nitrous oxide. Growth limiting factors for enterobacteria include pH < 4.5 to 5.0, a_w < 0.95 and temperature < 8^{o}C (O'Kiely and Muck, 1998). Enterobacterial activity is more likely where forage of low water soluble carbohydrate (WSC) concentration or high buffering capacity occur together with contamination of grass with sources of inoculum such as animal manure or soil. From among the Enterobacteria, *Escherichia coli* is particularly important because of the virulance of some strains towards livestock and humans. The objectives of the present experiment were to trace the development of a specific, non-infectious strain of *E. coli* 0157:H7 throughout silage fermentation, to determine its influence on the fermentation profile and to determine the effects of altering the ensilage conditions on *E. coli* 0157:H7 numbers.

Material and Methods

Unwilted, clean, precision-chop grass was ensiled (6 kg grass per silo) in laboratory silos (O'Kiely and Wilson, 1991) in mid June with the following treatment: (a) no additive

(control), (b) *E. coli* 0157:H7, (c) formic acid (850 g kg^{-1}; 3 ml kg^{-1} grass), and (d) *E. coli* 0157:H7 plus formic acid (i.e. (b) + (c)). A non-infectious strain of *E. coli* 0157:H7 was used, and was applied at log_{10} 4.5 colony forming units/g forage. Additives were applied manually immediately before ensiling and silos were stored at 15°C. There were 18 silos per treatment, three of which were opened 0, 2, 5, 9, 19 and 180 days post ensiling, and subjected to physical, chemical and microbiological analyses. Enterobacteria were recovered in VRBGA (Violet Red Bile Glucose Agar) while *E. coli* 0157:H7 were recovered on TSA (Tryptone Soya Agar – to recover injured cells) at 37°C for 2 hours and then overlayed with SMAC (Sorbitol MacConkey agar) to select for 0157. Forage dry matter (DM) concentration was determined after drying of samples at 40°C for 48 h in an oven with forced air circulation. Dried samples were milled through a sieve with 1 mm holes, with buffering capacity being determined according to Playne and McDonald (1966) and crude protein (N x 6.25) according to AOAC (1990) using a nitrogen analyser (LECO FP 428). Juice extracted from undried silage samples was assayed for lactic acid (Ciba-Corning Diagnostics 550 Express clinical chemistry analyser using the method of Boehringer Mannheim (Catalogue number 139004)), volatile fatty acids (Ranfft, 1973) and WSC (Wilson, 1978). Ammonia-N was measured by a modification of the method of O'Keeffe and Sherington (1983) and pH was determined using a combined reference and glass electrode. Data were analysed statistically by 3-way analysis of variance for a 2 (*E. coli*) x 2 (formic acid) x 6 (days of opening) factorial design.

Results and Discussion

Forage microbiological and chemical analysis data are summarised in Table 1. The low buffering capacity of the grass used in this experiment probably reflects its low crude protein concentration (Muck *et al.*, 1991), and compensated for the correspondingly low concentration of WSC to indicate a forage that was not difficult to preserve by ensilage. The

low buffering capacity also facilitated a rapid rate of drop in pH in response to lactic acid production. Silage made without additive duly had a relatively rapid rate of pH decline and underwent a lactic acid dominant fermentation. The mean concentration of crude protein was 100 g kg^{-1} DM and the concentrations of propionic and butyric acids and nitrates were each below 1 g kg^{-1} DM throughout. Numbers of indigenous Enterobacteria were high initially but declined progressively until colonies were no longer recoverable by day 19 of ensilage. This was reflected in the relatively low concentrations of acetic acid and ammonia-N found in the control silage during the early stages of ensilage. No indigenous *E. coli* 0157:H7 appeared to be present at any stage of ensilage. Grass ensiled with or without formic acid provided contrasting ensiling conditions. Formic acid restricted fermentation, reducing ($P<0.001$) buffering capacity and the concentration of lactic acid and increasing ($P<0.001$) the concentration of WSC. Formic acid addition increased ($P<0.05$) the initial rate of decline in enterobacterial numbers. This probably reflected both its immediate effect of reducing forage pH together with the specific antibacterial effect of the undissociated acid (McDonald *et al.*, 1991). Inoculation of forage with *E. coli* 0157:H7 elevated ($P<0.001$) the numbers of *E. coli* 0157:H7 present on the forage. The latter numbers declined rapidly and appeared to have disappeared by day 5 of ensilage. That *E. coli* 0157:H7 cfu dropped to zero sooner than for Enterobacteria may have been largely due to the substantially higher counts of the indigenous Enterobacteria compared to the inoculated *E. coli* bacteria at the start of ensilage, as well as to possible differences in their relative tolerance of the prevailing ensilage conditions. The addition of formic acid accelerated ($P<0.001$) this rate of decline after day 0 of ensilage. Inoculation of forage with *E. coli* 0157:H7 did not alter ($P>0.05$) silage fermentation or the rate of decline in the number of colonies of Enterobacteria detected. This is not surprising given the speed with which conditions within the silo became inhibitory for the inoculated *E. coli*.

The above results agree with a previous study (O'Kiely *et al.*, 1999) in which the effects of inoculating grass with *E. coli* 0157:H7 at ensiling were quantified during the first ten days of ensilage. Due to the rapid rate of pH decline in both studies, there remains the need to determine the survival of *E. coli* 0157:H7 during ensilage and its influence on silage fermentation when it has been inoculated onto grass of high buffering capacity and relatively low WSC concentration, and possibly at higher numbers than were used in this experiment.

In conclusion, the number of colonies of both inoculated *E. coli* 0157:H7 and indigenous Enterobacteria decreased rapidly to undetectable levels where unwilted precision-chopped grass was ensiled and underwent a rapid, lactic acid dominant fermentation. Altering the ensiling conditions by adding formic acid increased the rate of decline of both *E. coli* 0157:H7 and Enterobacteria. Under these conditions, inoculation of grass at ensiling with *E. coli* 0157:H7 did not influence the fermentation during 180 days of ensiling.

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Table 1 - Forage chemical and microbiological composition throughout ensilage.

	Dry matter (DM) (g kg^{-1})	pH	NH_3-N (g kg^{-1} total N)	Lactic acid (g kg^{-1} DM)	Acetic acid (g kg^{-1} DM)	WSC[1] (g kg^{-1} DM)	Buffering capacity (mEq. kg^{-1} DM)	*E. coli* (log_{10} CFU[2] g^{-1})	Enterobacteria (log_{10} CFU[2] g^{-1})
Day 0									
No additive	197	4.40	32	14	10	89	331	0	8.12
E. coli (Ec.)	205	4.40	33	12	9	98	330	3.60	7.80
Formic acid (F.A.)	207	4.23	15	1	4	111	289	0	6.32
E.c. + F.A.	205	4.20	14	1	2	115	293	3.53	6.48
Day 2									
No additive	194	4.00	28	54	10	57	432	0	5.13
E. coli	197	4.00	28	56	13	52	394	3.49	5.16
Formic acid	201	4.03	14	11	1	94	357	0	4.67
E.c. + F.A.	202	4.07	22	14	3	84	316	2.55	4.85
Day 5									
No additive	197	3.83	41	79	11	45	467	0	3.34
E. coli	197	3.80	39	77	11	38	465	0	4.37
Formic acid	195	4.00	24	22	4	71	338	0	3.96
E.c. + F.A.	193	4.00	25	24	3	68	335	0	3.12
Day 9									
No additive	191	3.70	44	105	16	28	596	0	1.71
E. coli	192	3.70	42	103	15	25	596	0	0.38
Formic acid	187	3.83	31	45	8	36	446	0	1.61
E.c. + F.A.	179	3.80	34	52	8	31	447	0	0.56
Day 19									
No additive	194	3.60	47	111	17	21	653	0	0
E. coli	197	3.60	47	110	19	23	627	0	0
Formic acid	187	3.73	35	56	42	29	463	0	0
E.c. + F.A.	184	3.73	32	62	46	28	459	0	0
Day 180									
No additive	172	3.70	50	129	21	16	668	0	0
E. coli	173	3.70	51	132	20	16	721	0	0
Formic acid	175	3.73	45	88	14	24	638	0	0
E.c. + F.A.	172	3.67	47	92	16	20	666	0	0
s.e.m. (E.c.xFAxday)	7.4	0.033	1.7	3.1	1.7	3.3	15.3	0.054	0.428
Significance									
E.c.	NS	NS	NS	NS	NS	NS	NS	***	NS
F.A.	NS	***	***	***	*	***	***	***	*
E.C. x F.A.	*	NS	NS	NS	NS	NS	NS	***	NS
Day	***	***	***	***	***	***	***	***	***
E.c. x day	NS	NS	NS	NS	NS	*	NS	***	NS
F.A. x day	***	***	***	***	***	***	***	***	NS
E.c. x F.A. x day	NA	NS	NS	NS	NS	NS	NS	***	NS

[1]WSC = water soluble carbohydrates [2]CFU = colony forming units