Effect of bacterial inoculants on fermentation end-products and *in vitro* digestibility of corn silage: a meta-analysis over a 37-year period

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Keywords dry matter digestibility, lactic acid, silage inoculant

Introduction Bacterial inoculants comprised of lactic acid bacteria (LAB) are used as silage additives worldwide. Whole-crop corn is known to possess properties that can make good silage through a natural fermentation process; therefore, utilization of homofermentative LAB (^{ho}LAB) inoculation is questionable for this crop. Further investigation of ^{mix}LAB inoculation (i.e., inoculation with both ^{ho}LAB and heterofermentative LAB - ^{he}LAB) on fermentation profile and nutritive value of corn silage is required to know a possible synergistic effect. Moreover, the results found in literature regarding fermentation profile and *in vitro* digestibility of corn silage following silage inoculation is inconsistent in some cases. Thus, inoculation of corn silage with different LAB on fermentation end-products and *in vitro* digestibility was examined through a meta-analysis approach.

Material and methods A database containing 141 articles published in journals (731 treatment means evaluated) was used to examine fermentation end-products and *in vitro* digestibility of corn silage. The main inclusion criteria for the database were: 1) comparison of one or more bacterial inoculants against a negative control (i.e., untreated forage - control); 2) description of bacteria's species used for silage inoculation; 3) description of the application rate for silage inoculation; 4) length of fermentation reported; 5) results regarding fermentation patterns and *in vitro* digestibility. Treatments were classified into the following categories: 1) silage with no inoculant (control), 2) silage treated with homolactic and facultative heterolactic LAB (^{ho}LAB: *Lactobacillus acidophilus*, *L. curvatus*, *L. paracasei*, *L. plantarum*, *L. salivarius*, *Enterococcus faecium*, *Pediococcus acidilactici* and *P. pentosaceus*, or their combinations), 3) silage treated with obligate heterolactic LAB (^{he}LAB: *L. brevis* and *L. buchneri*, or their combinations), and 4) silage treated with both ^{ho}LAB and ^{he}LAB (^{mix}LAB). Data were analyzed as mixed models using the MIXED procedure of SAS (v. 9.4). Study effect was considered to be a random effect and was included in the model using the RANDOM statement (St-Pierre, 2001). Moreover, the inverse of the squared standard error of each treatment mean or the inverse of the number of observations of each study (when squared standard error was lacking) were used as a factor in the WEIGHT statement of the model. The application rate of bacterial inoculation, storage temperature and the type of silo (laboratory- and farm-scale silos) were used as covariates. However, if the random covariance was not significant, they were removed from the model if $P > 0.05$ (St-Pierre, 2001). Differences between means were determined using the P-DIFF option of the LSMEANS statement at $P \leq 0.05$.

Results and discussion The current study confirmed that inoculation of corn silage with ^{ho}LAB increased ($P < 0.01$) lactic acid concentration (+37.4%; Table 1). As lactic acid is the strongest acid found within the silo, the final pH of corn silage inoculated with ^{ho}LAB was reduced ($P < 0.01$). Inoculation with ^{ho}LAB reduced the ammonia-N concentration ($P = 0.01$) of corn silage by 8%, likely by reducing the clostridia population (data not shown) which has proteolytic activity. In contrast to the reduced acetic acid in the ^{ho}LAB silage, corn silages inoculated with ^{he}LAB exhibited higher ($P < 0.01$) concentration of acetic acid (+49.6% compared to the control silage) and 1,2-propanediol, and lower concentration of lactic acid. During the

fermentation process, ^{he}LAB convert water-soluble carbohydrates to acetic acid, and some ^{he}LABs (e.g., *L. buchneri* and *L. brevis*) have the ability to convert lactic acid into equal molar proportions of acetic acid and 1,2-propanediol under anaerobic conditions (Oude Elferink et al., 2001). Ethanol concentration was unaffected (P = 0.11) by corn silage inoculation. Inoculation of corn silage resulted in improved *in vitro* neutral detergent fiber (NDF-D; P < 0.01), regardless of the inoculant used. Certain ^{he}LAB strains were found to have the ability to produce the enzyme feruloyl esterase, which may explain the increased NDF-D observed in ^{he}LAB and ^{mix}LAB silages. Conversely, we do not have a clear explanation regarding the increased NDF-D in the ^{ho}LAB. Increased fiber digestion is expected to positively affect DM digestibility, however, only silages inoculated with ^{ho}LAB exhibited higher *in vitro* dry matter digestibility (IVDMD; P < 0.01). This result is likely associated with the lower acid detergent fiber concentration (data not shown) reported in ^{ho}LAB silage as compared with that in the control.

Table 1 Effects of lactic acid bacteria inoculation on fermentation end-products and *in vitro* digestibility of corn silage.

Item*	Control	Silage inoculant[1]			*P*-value
		^{ho}LAB	^{he}LAB	^{mix}LAB	
Fermentative profile, g/kg DM					
pH	3.81^{a} ± 0.02	3.75^{b} ± 0.02	3.84^{a} ± 0.03	3.82^{a} ± 0.02	<0.01
Ammonia-N, g/kg TN	45.8^{a} ± 5.98	42.1^{b} ± 5.96	44.3^{a} ± 5.96	43.5^{a} ± 6.00	0.01
Lactic acid	42.2^{b} ± 2.75	67.4^{a} ± 2.75	28.6^{c} ± 2.85	47.1^{b} ± 3.32	<0.01
Acetic acid	13.7^{c} ± 1.12	12.5^{d} ± 1.16	27.2^{a} ± 1.45	17.5^{b} ± 1.46	<0.01
Butyric acid	0.34^{b} ± 0.11	0.33^{b} ± 0.11	0.76^{a} ± 0.17	0.40^{b} ± 0.12	0.03
Ethanol	9.04 ± 1.39	8.91 ± 1.33	8.35 ± 1.33	8.51 ± 1.37	0.11
1,2-propanediol	1.53^{c} ± 0.04	0.42^{d} ± 0.26	4.50^{b} ± 0.90	10.0^{a} ± 1,37	<0.01
In vitro digestibility, g/kg					
DM	632^{b} ± 18.0	646^{a} ± 18.0	608^{b} ± 21.4	624^{b} ± 24.9	<0.01
NDF	420^{b} ± 24.8	460^{a} ± 23.0	473^{a} ± 24.6	464^{a} ± 27.4	0.01

$^{a-c}$Means in the same row with different superscripts differed significantly ($P \leq 0.05$).
[1]Control = corn silage with no inoculant; ^{ho}LAB = corn silage treated with homolactic and facultative heterolactic bacteria at the application rates from 4.3×10^{2} to 1×10^{11} cfu/g of fresh forage; ^{he}LAB = corn silage treated with obligate heterolactic bacteria at the application rates from 1×10^{3} to 7×10^{8} cfu/g of fresh forage; and ^{mix}LAB = corn silage treated with obligate heterolactic bacteria at the application rates from 3.4×10^{4} to 1×10^{8} cfu/g of fresh forage.
*DM = dry matter; TN = total nitrogen; NDF = neutral detergent fiber.

Conclusion This meta-analysis provided a quantitative summarization of 37-yrs period and indicated, in general, that the fermentation end-products of corn silage may differ owing the type of LAB used at inoculation, but IVDMD is increased only by inoculating ^{ho}LAB.

References

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