



# Forage fermentation product measures are related to dry matter loss through meta-analysis

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## ABSTRACT

Forage preservation through fermentation can result in substantial DM and economic losses. The objective of this research by the use of meta-analysis was to determine whether fermentation end-product measures and forage parameters were capable of predicting forage DM losses following ensiling. The data set was built by searching a database for “forage,” “fermentation,” and “dry matter loss.” The data set contained 405 means from 43 peer-reviewed research reports. Forage DM losses (% of original forage DM) ranged from 0 to 28.6% with a raw mean of 6.2%. Report, forage biology, fermentation treatment, fermentation length, DM, pH, lactic acid, and acetic acid parameters were related to natural logarithm DM loss using a mixed-model approach. Parameters were evaluated for linear and quadratic effects and linear interactions. Report was classified as a random effect. The resulting model had a 1.403 mean natural logarithm DM,  $R^2$  of 0.813, and root mean square error of 0.418. Forage DM (%), acetic and lactic acid, pH, fermentation

length, and forage biology were related to losses. Fermentation treatments tended ( $P < 0.10$ ) to differ. Forage pH  $\times$  lactic acid, DM  $\times$  fermentation treatment, DM  $\times$  forage biology, and lactic acid  $\times$  forage biology all exhibited interactions. Forage DM, biology, fermentation, and treatment parameters were capable of describing most DM-loss variation across a range of published research reports ( $P \leq 0.01$ ). The final model described here has utility to predict forage DM losses due to fermentation and may be useful to diagnose problematic fermentations and assess opportunity costs.

**Key words:** dry matter loss, efficiency, fermentation, forage, preservation

## INTRODUCTION

Forages preserved through fermentation are used to provide feed for ruminant animals. Ensiled feedstuffs total 132 million tonnes in the United States (NASS, 2007). Fermentation losses range from 3 to 25% of DM (Pitt, 1986). Applying the median DM loss (14%) to yearly harvested forage suggests at least 18 million tonnes of DM are lost each year. Using \$152 per tonne of forage, adapted

from Cabrera et al. (2014), losses in the United States equate to approximately \$3 billion annually. However, estimating potential DM losses on farm is difficult.

The aim in ensiling feedstuffs is to yield a homolactic or heterolactic anaerobic fermentation. Fermenting microorganisms grow, metabolizing water-soluble carbohydrates into organic fermentation acids (Weinberg and Muck, 1996). Lactic, acetic, butyric, and propionic acid are major acids produced during ensiling. The dissociation constants (Nelson and Cox, 2003) are 3.86 for lactic acid, 4.76 for acetic acid, 4.82 for butyric acid, and 4.87 for propionic acid. A lower dissociation constant signifies stronger acid; hence, lactic acid is a stronger acid and a primary factor for decreased ensiled-forage (silage) pH.

Weinberg and Muck (1996) characterized fermentation into 4 stages: (1) aerobic, (2) fermentation, (3) stable, and (4) feed out. After ending the aerobic phase (stage 1), anaerobic microbial growth and fermentation acids increase (stage 2) until forage pH decreases to the point that microbial activity slows. Approximate pH ranges at silage stability are 3.5 to 4.5 for corn- and grass-based silages and

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4.0 to 5.0 for legumes. At this point the forage is effectively preserved (stage 3) until feedstuffs are again exposed to oxygen (stage 4). Forage DM losses can take place at each of these 4 stages, but the focus of this research is to determine whether losses can be characterized for stages 1, 2, and 3. Stage 4 losses can also be substantial but relate to aerobic microbial growth, spoilage, and subjective silage disposal by farm owners due to spoilage.

Regression models have been developed to predict fermentation acid results (Pitt et al., 1985; Mogodiniyai Kasmaei et al., 2013); however, these models did not offer insight into DM losses. Pitt (1986) developed a mathematical model to predict DM losses due to oxygen but did not incorporate other fermentation parameters. The objective of this research was to determine, by meta-analysis, whether forage and fermentation parameters are capable of predicting forage DM losses following ensiling.

## MATERIALS AND METHODS

### *Online Database Query and Meta-Analysis Database Build*

In May 2013 the Web of Knowledge (www.webofknowledge.com) database was queried with “forage,” “fermentation,” and “dry matter loss” key words for peer-reviewed research reports containing forage fermentation measurements and corresponding DM loss measures. Forage DM loss is defined as the remaining forage DM (weight) after ensiling relative to original DM ensiled. Each article was then individually reviewed to identify those that reported DM loss. This data set was further refined to include only those in which reports included pH, lactic acid, acetic acid, butyric acid, propionic acid, ethanol, or ammonia-N. Lactic acid and acetic acid measures were reported in all but one (report 35). Reports and treatment means in the final data set included minisilo (defined as less than 10 kg of fresh forage per silo,  $n = 337$  treatment means), macrosilo (greater than 10

kg but not a farm silo,  $n = 45$ ), and farm-scale silos (forage ensiled in an on-farm storage structure, including wrapped bale,  $n = 23$ ). Silo type was evaluated using both box plot and the effect-screening, model-building function within SAS JMP Pro Version 11 (SAS Institute Inc., Cary, NC), finding silo not related to DM loss ( $P < 0.10$ ).

The final data set incorporating published treatment means was created, with report identified by a unique numeric ( $n = 1$  to 43). Forage type was categorized by plant physiology and entered as legume ( $n = 59$  treatment means), C3 grass ( $n = 171$ ), C4 grass ( $n = 95$ ), C3 grass-legume mix ( $n = 60$ ), or other ( $n = 20$ ). Forage-specie details can be found within references listed in the Appendix.

Fermentation treatments were categorized into 5 classes: untreated (control,  $n = 184$ ), inoculant with lactic acid-producing bacteria not containing *Lactobacillus buchneri* ( $n = 116$ ), bacterial inoculant containing *Lactobacillus buchneri* ( $n = 24$ ), bacterial inoculant containing both of the previous inoculants ( $n = 28$ ), or other forage-preservation aid ( $n = 53$ ). Other forage-preservation aids included formic or propionic acid, sodium benzoate, potassium sorbate, and sodium nitrite.

Fermentation length (days) was entered as a continuous variable for all treatment means except for report 17 ( $n = 4$  treatment means). Fermented-forage treatment means for DM ( $n = 391$  treatment means), CP ( $n = 217$ ), NDF ( $n = 163$ ), starch ( $n = 32$ ), ash ( $n = 101$ ) and water-soluble carbohydrates ( $n = 263$ ) were entered as reported on a DM basis as continuous variables.

Lactic acid, acetic acid, butyric acid, propionic acid, and ethanol content were entered into the data set on a percentage of DM basis. Ammonia-N ( $\text{NH}_3\text{-N}$ ) was either entered as percentage of CP or converted to percentage of CP by dividing reported value (% of DM) by forage CP content (DM basis). For report 5, 20 treatment means did not report a corresponding CP value. In this case, to

calculate  $\text{NH}_3\text{-N}$  (% of CP), grass and legume silages were assumed 14 and 17% CP (DM basis), respectively.

In total, 43 peer-reviewed research reports containing 405 treatment means were identified that reported measures of both DM loss and fermentation compound. Although additional studies likely exist beyond those identified here that report these measures, we assume that those identified represent the population of results published, without bias. Reports included within the meta-analysis are listed within the Appendix, and descriptive statistics relating to data entered are presented in Table 1.

### *Statistical Analysis*

The meta-analysis data set included 405 treatment means. Reports, forage biology, fermentation treatment, fermentation length, DM, pH, lactic acid, and acetic acid parameters were chosen for evaluation using a regression-model approach. These parameters were reported in entirety for most treatment means ( $n = 391$ ). Linear and quadratic parameter effects were evaluated for continuous variables. Treatment DM loss and natural logarithm DM (DMnl) loss means were considered dependent variables.

All statistical analyses were carried out using SAS JMP Pro Version 11 (SAS Institute Inc.). Dry matter loss means were first regressed against model parameters using backward elimination through JMP mixed modeling. The backward elimination was completed by beginning with a full model, including interactions, and sequentially removing nonsignificant parameters while also evaluating Akaike's information criterion and the Bayesian information criterion for best model fit, with lower Akaike's information criterion and the Bayesian information criterion assumed superior. Parameters outlined previously as well as all 2-way interactions were assessed within model. All parameters were considered fixed except for report, which was identified as a random effect under the strategy described by St-Pierre (2001). The

**Table 1. Descriptive statistics for parameters included within meta-analysis**

| Item   | n   | Mean | SD   | Minimum | Maximum |
|--|-----|------|------|---------|---------|
| Forage physiology  | 405 |      |      |         |         |
| Fermentation treatment                                   | 405 |      |      |         |         |
| Fermentation length, d                                   | 398 | 84.7 | 67.0 | 1.0     | 575.0   |
| DM   | 391 | 28.6 | 9.9  | 11.0    | 69.4    |
| Fermented forage nutritive measures, % of DM             |     |      |      |         |         |
| CP   | 217 | 13.4 | 5.7  | 1.5     | 25.5    |
| NDF  | 163 | 51.5 | 9.8  | 31.3    | 80.8    |
| Starch   | 32  | 24.4 | 7.0  | 9.2     | 34.2    |
| Water-soluble carbohydrates                              | 263 | 4.71 | 4.3  | 0.0     | 19.8    |
| Ash  | 101 | 9.89 | 3.8  | 3.2     | 19.0    |
| pH   | 405 | 4.24 | 0.5  | 3.1     | 7.1     |
| Fermentation compounds, % of DM, unless otherwise listed |     |      |      |         |         |
| Lactic acid  | 400 | 5.78 | 3.3  | 0.2     | 17.3    |
| Acetic acid  | 391 | 1.93 | 1.6  | 0.0     | 12.2    |
| Butyric acid   | 218 | 0.44 | 1.0  | 0.0     | 7.2     |
| Propionic acid   | 140 | 0.11 | 0.2  | 0.0     | 1.2     |
| Ethanol  | 226 | 1.50 | 2.3  | 0.0     | 17.2    |
| Ammonia-N, % of CP                                       | 346 | 8.42 | 5.5  | 0.5     | 29.2    |
| Calculated total acid                                    | 405 | 7.85 | 3.9  | 0.0     | 25.4    |
| Yeast count, log cfu/g                                   | 86  | 2.17 | 1.4  | 0.0     | 5.7     |
| Yeast count, log cfu/g                                   | 92  | 2.28 | 1.5  | 0.0     | 7.1     |
| Clostridial spores, log cfu/g                            | 33  | 1.12 | 1.0  | 0.5     | 4.5     |
| DM loss, % of fresh forage DM                            | 405 | 6.2  | 5.2  | 0.0     | 28.6    |

model was fit with JMP Pro Version 11 mixed-model personality and study identified as a random effect, allowing both study intercept and slope to differ under this approach.

Following initial modeling using DM loss as the dependent variable, a new data column with residuals was created and residuals were assessed for normality using Shapiro and Wilk (1965) goodness-of-fit test. Dry matter loss data distribution was found to be significantly different from normal ( $P < 0.0001$ ). As a result, DM loss data were transformed using natural logarithm function and the entire model-fit procedure was repeated as described previously, using backward elimination, and parameters or interactions with  $P < 0.10$  were retained. Residual plot following transformation is shown in Figure 1.

With DMnl loss transformation, final model fit residual distribution was not different from normal. Model Akaike's information criterion and

Bayesian information criterion were compared when the final parameter was removed from the model, and smaller Akaike's information criterion and Bayesian information criterion was used to aid in final model selection. Significance was declared at  $P < 0.05$  and tendency toward significance at  $P < 0.10$ . The final model was

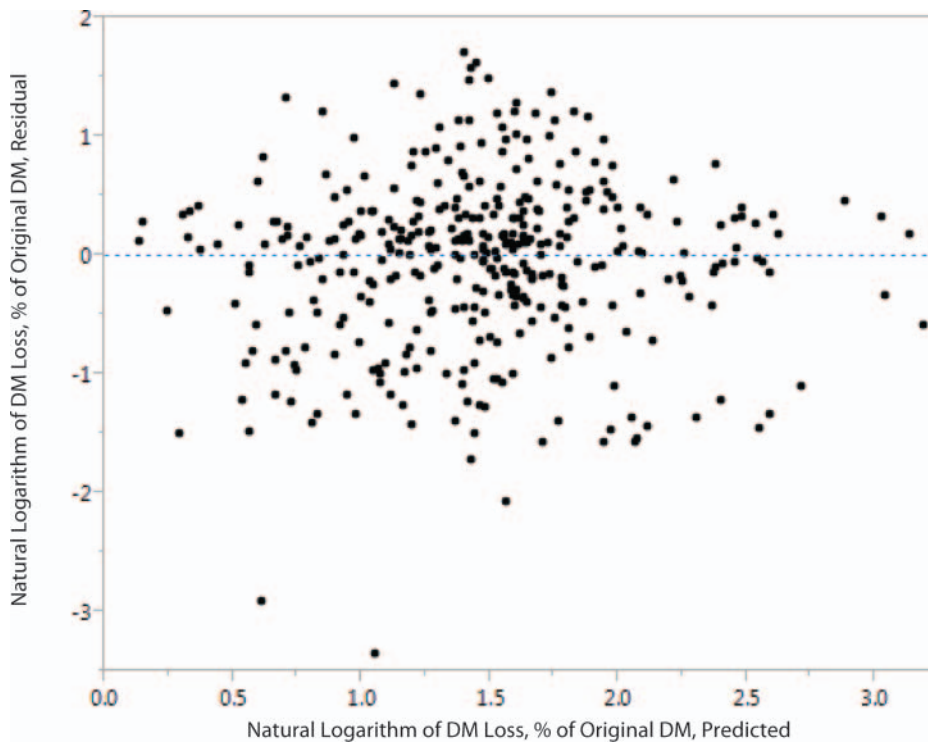
$$Y_{ijklmnop} = \mu + D_i + A_j + F_k + L_l + P_m + G_n + B_o + S_p + DF_{ik} + PL_{ml} + DB_{io} + LB_{lo} + e_{ijklmnop}$$

where  $Y_{ijklmnop}$  = DMnl loss, the dependent variable,  $\mu$  = population mean,  $D_i$  = fixed effect of DM (preensiling),  $A_j$  = fixed effect of acetic acid,  $F_k$  = fixed effect of fermentation treatment,  $L_l$  = fixed effect of fermentation length,  $P_m$  = fixed effect of pH,  $G_n$  = fixed effect of lactic acid,  $B_o$  = fixed effect of forage biology,  $S_p$  = random effect of report,  $DF_{ik}$  = DM and fermentation treatment interaction,  $PL_{ml}$

= pH and lactic acid interaction,  $DB_{io}$  = DM and forage biology interaction,  $LB_{lo}$  = lactic acid and forage biology interaction, and  $e_{ijklmnop}$  = random residual error, assumed to be normally distributed.

## RESULTS AND DISCUSSION

Forage DM losses during ensiling result from bacterial substrate degradation and carbon dioxide production (Pitt, 1986). Resulting forage DM losses within the meta-analysis data set averaged 6.2% and ranged from 0 to 28.6% of DM (Table 1), similar to the range reported by Pitt (1986). After DMnl transformation, the final regression model discussed here exhibited a 1.403 mean DMnl loss response (DM loss equivalent of 4.07%), accounted for more than 81% of total variation within the meta-analyses data set (adjusted  $R^2 = 0.813$ ), and revealed a root mean square error of 0.418. Several different silo types were



**Figure 1.** Natural logarithm of DM loss final-model residuals plotted against predicted values. Color version available online.

included within the data set evaluated in this meta-analysis, with possible differences in forage density and oxygen exposure. Forage density (Ruppel et al., 1995) and resulting oxygen (Pitt, 1986) infiltration affect the fermentation process, yet the stoichiometry of forage DM metabolization into carbon dioxide and fermentation acid or other compounds such as alcohols was assumed similar across silo types, densities, and oxygen exposures. The final model regression parameters for all variables in relation to DM loss (% of sample DM) are presented in Table 2.

Muck (1988) summarized 3 processes as being responsible for excessive DM and energy losses similar to that investigated here. These processes included plant respiration, aerobic microorganism growth, and clostridia growth. The parameters related to DM losses presented here likely result from one or a combination of the 3 aspects described. Regression parameters will be discussed in order relating to respective significance level. Oxygen infiltration, related to each of the 3 processes Muck (1988)

described, has further been implicated to account for a great amount for DM losses (Pitt, 1986); however, it was not directly accounted for here. Rather, fermentation outcome was used to describe DM losses.

Following mixed-model analysis, pH was positively ( $P < 0.0001$ ) related to DM losses ( $y = \text{intercept} + 0.740 \times \text{pH}$ ). Our observation agrees with Muck (1988), who described that forage pH decline is largely responsible for stabilizing fermentation and slowing continued microbial activity and extensive DM losses. As described previously, lactic acid production is a primary factor leading to decreased forage pH because of the lower dissociation constant relative to other fermentation products.

Forage DM content was negatively ( $y = \text{intercept} - 0.036 \times \text{DM content, \%}$ ) related to DM losses following fermentation. Forage DM content has been related to plant protease activity (Muck, 1988), seepage losses (Holter, 1983), and secondary or clostridial fermentation (Weinberg and Muck, 1996). Increases in each of these processes can lead to increased

forage losses; however, seepage losses were likely not a factor within this meta-analysis because of sealed silos reported for most treatments within the database used. Our results agree with Yahaya et al. (2002), where greater-moisture (76%) orchardgrass resulted in significantly greater water-soluble carbohydrate, hemicellulose, and energy losses relative to lesser moisture (40 or 65%). Our results differ, however, with those reported by Ruppel et al. (1995), who found no relationship between forage DM content and DM losses on dairy farms. The number of observations in this meta-analysis as well as the difference in silo sizes and scale may explain the difference between our observations and those observed by Ruppel et al. (1995).

Based on slope estimates, as DM content decreased, losses were generally greater (negative or zero slope estimates) except for forage-biology other (including both water hyacinth and safflower silages, Table 2), which contributed to the interaction observed for DM content and forage biology (Table 2,  $P < 0.0006$ ). This observation would suggest that other forage types were not demonstrating greater losses at lesser DM contents and warrants further evaluation.

Further evaluation of the DM-by-forage biology interaction showed that C4-classified-grasses losses increased to a greater extent than other forage classes as DM lessened, evidenced by a significantly negative slope. This observation may suggest that at lesser DM concentrations, C4 grasses undergo more prolonged fermentation and ensiling organisms likely consume more substrate before fermentation stabilizes. Hence, decreased forage DM should be avoided in C4 grasses.

We also observed an interaction between forage DM content and forage treatment, likely due to different forage-treatment slopes when relating DM content to DMnl losses (Table 2). Fermentation-aid categorized treatments appeared to relate to greater forage recovery (negative slope estimate) as DM increased relative to other forage-treatment categories.

**Table 2. Parameter estimates, SE, and significance level for continuous variables in relation to natural logarithm of DM loss following mixed-model multiple-linear-regression analysis<sup>1</sup>**

| Model parameter  | Estimate | SE    | P-value |
|--|----------|-------|---------|
| Intercept  | -0.973   | 0.635 | 0.127   |
| DM, %  | -0.036   | 0.007 | <0.0001 |
| Acetic acid, % of DM   | 0.093    | 0.028 | 0.0009  |
| Fermentation length, d   | 0.002    | 0.001 | 0.0188  |
| pH   | 0.740    | 0.121 | <0.0001 |
| Lactic acid, % of DM   | 0.349    | 0.095 | 0.0003  |
| pH × lactic acid, % of DM  | -0.088   | 0.022 | <0.0001 |
| Fermentation treatment   |          |       |         |
| Control  | 0.231    | 0.186 | 0.2148  |
| Lactic acid-producing bacterial inoculant not containing <i>Lactobacillus buchneri</i> (LAB) | 0.080    | 0.206 | 0.6981  |
| Bacterial inoculant containing <i>Lactobacillus buchneri</i> (BUCH)                          | -0.604   | 0.422 | 0.1529  |
| Bacterial inoculant containing both LAB and BUCH (COM)                                       | -0.476   | 0.399 | 0.2334  |
| Other forage-preservation aid  | 0.769    | 0.288 | 0.0080  |
| Forage biology   |          |       |         |
| C3 grass   | -0.318   | 0.279 | 0.2554  |
| Mixed forage: C3 grass and legume  | -0.312   | 0.455 | 0.4933  |
| C4 grass   | 2.036    | 0.543 | 0.0002  |
| Legume   | -0.225   | 0.311 | 0.4703  |
| Other species  | -1.180   | 0.536 | 0.0300  |
| DM, % × fermentation treatment interaction   |          |       |         |
| DM × control   | -0.002   | 0.006 | 0.7139  |
| DM × LAB   | -0.002   | 0.006 | 0.7978  |
| DM × BUCH  | 0.024    | 0.013 | 0.0600  |
| DM × COM   | 0.011    | 0.013 | 0.3673  |
| DM × other forage-preservation aid   | -0.032   | 0.011 | 0.0027  |
| DM (%) × forage biology interaction  |          |       |         |
| DM × C3 grass  | -0.003   | 0.007 | 0.6909  |
| DM × mixed forage: C3 grass and legume   | 0.010    | 0.013 | 0.4401  |
| DM × C4 grass  | -0.048   | 0.015 | 0.0014  |
| DM × legume  | 0.006    | 0.008 | 0.4197  |
| DM × other species   | 0.034    | 0.009 | <0.0001 |
| Lactic acid, % of DM × forage biology interaction  |          |       |         |
| Lactic acid × C3 grass   | 0.064    | 0.018 | 0.0003  |
| Lactic acid × mixed forage: C3 grass and legume  | 0.003    | 0.024 | 0.8950  |
| Lactic acid × C4 grass   | -0.123   | 0.030 | <0.0001 |
| Lactic acid × legume   | -0.016   | 0.024 | 0.5145  |
| Lactic acid × other species  | 0.072    | 0.039 | 0.0672  |

<sup>1</sup>Final prediction model exhibited a 1.403 mean, adjusted R<sup>2</sup> = 0.813, and root mean square error of 0.418.

This observation may be due to lower water activity at greater DM contents and less-efficient bacterial growth (Leibensperger and Pitt, 1987) and less subsequent fermentation-acid production by bacterial inoculants. In the absence of substantial bacterial-acid production, forage-preservation-aid treatments (including formic or propionic acid, sodium benzoate, potassium sorbate, or sodium nitrite) can act to inhibit undesirable microbial growth and limit DM losses. Woolford

(1975) evaluated the effect of various food preservatives, such as potassium sorbate, and chemical compounds, such as sodium nitrite, on silage and found several different preservation aids suppressed bacterial, yeast, and mold growth. These preservative aids appear to mitigate DM losses across a greater DM range than bacterial inoculants or control approaches.

After accounting for pH within regression model, greater lactate and acetic acid concentrations were re-

lated to increased DM losses. Individual fermentation-acid slope estimates were 0.349 and 0.093 for lactic and acetic acids, respectively, in relation to DMnl loss (Table 2). Whereas the aim in fermentation preservation is to rapidly decrease pH through a homo- or hetero-lactic fermentation process, substrate must be degraded to yield acidic end products. Though it is possible to completely conserve forage DM through bacterial conversion of glucose to lactic acid (Savoie and

Jofriet, 2003), likely as substantial amounts of lactic acid are produced during fermentation, other metabolism pathways ensue, where glucose is metabolized into gases and water in addition to lactic acid, possibly explaining the positive relationship between lactic acid and DMnl loss.

Logically, as lactic acid is produced, pH decreases in forage. However, the 2 main effects were inversely related to DMnl loss, and we further observed a pH-by-lactic acid interaction (Table 2). The continuous interaction can be described as a different DMnl loss response in relation to pH or lactic acid depending on lactic acid or pH levels. In further describing the interaction, at relatively low pH levels (<4.25), lactic acid appeared to follow the main effect relationship with DMnl loss, namely greater lactic acid was related to increased DMnl loss. This may be explained through the process described previously where substantial glucose was metabolized into water and gasses, possibly under extended fermentation where pH resulted at a low level but fermentation took substantially longer to stabilize.

However, at greater pH levels, for example with forage containing substantial buffering capacity or poor resulting fermentation, lactic acid appeared to have a negative relationship with DMnl loss. A possible interpretation of this observation may be that lactic acid load helped limit DM losses under challenged fermentation circumstances.

The positive linear relationship between acetic acid and DM loss is in agreement with Weinberg and Muck (1996), who outlined that acetic acid-producing bacterial activity results in substantial nutritive and DM losses. Beyond the metabolic pathways described previously where glucose is inefficiently converted to fermentation acids, appreciable acetic acid levels appear to indicate an inefficient fermentation. A possible reason being, in the absence of adequate pH decline, alternative fermentation microbes continue thriving and metabolizing water-soluble carbohydrates or other simple feed nutrients while producing acetic acid, gases, water, and other fermentation compounds. Forage pH will continue declining with acetic acid production; however, greater relative acetic acid levels are needed to decrease pH and stabilize fermentation compared with lactic acid.

Fermentation length, defined as days before silo opening, was positively related to DM losses ( $y = \text{intercept} + 0.002 \times \text{fermentation length, d}$ , Table 2). Our results agree with those of Yahaya et al. (2001) where the authors observed water-soluble carbohydrate, pectin, and hemicellulose degradation increased with longer fermentation times to 56 d. The fermentation-length relationship with DM losses is rational considering that bacteria and other microbes can continue metabolizing water-soluble sugars and other forage substrate such as pectins or hemicellulose until pH

decreases to the point that microbial activity slows and fermentation stabilizes. This response may have been expected to become quadratic at relatively longer fermentation length, given that successfully preserved forage eventually stabilizes and DM losses should cease. However, as described previously, quadratic main effects were not significant. The lack of a quadratic response may be due to limited fermentation length (mean of 85 d, SD of 67 d, Table 1) of treatment means used within this research.

Forage biology was related to DM losses ( $P < 0.006$ ), and slope estimates are presented in Table 2. On average C4 grasses (including whole-plant corn, corn stover, millet, sugarcane, and sorghum silages) demonstrated a greater slope estimate relative to other forage-biology categories; however, as mentioned previously, C4 grasses appeared non-linear in relation to DMnl loss as DM increased. Despite differing slope estimates, forage-biology categories were not different when evaluating least squares means comparisons (Table 3).

Forage-biology classes also differed in DM losses relative to lactic acid concentration (Table 2). Legumes and mixed forages containing legumes did not differ in DM losses as lactic acid levels changed. These differences may be due to differences in buffering capacity for legumes. Muck (1988) described that substrate necessary to decrease forage pH was dependent on both DM content and crop buffering

**Table 3. Forage-biology least squares natural logarithm of DM loss (% of DM) means and connecting letters report**

| Forage biology <sup>1</sup> | Least squares mean natural Log DM loss, % of DM | SE    | DM loss least squares mean, exp(Log DM loss) |
|-----------------------------|---|-------|--|
| Other species               | 1.54 <sup>a</sup>                               | 0.490 | 4.66   |
| C3 grass-legume mix         | 1.32 <sup>a</sup>                               | 0.156 | 3.75   |
| C3 grass                    | 1.29 <sup>a</sup>                               | 0.132 | 3.64   |
| C4 grass                    | 1.28 <sup>a</sup>                               | 0.159 | 3.59   |
| Legume                      | 1.19 <sup>a</sup>                               | 0.167 | 3.28   |

<sup>a</sup>Means with similar superscripts do not differ ( $P < 0.05$ ).

<sup>1</sup>Forage biology was categorized by plant physiology and entered as legume, C3 grass, C4 grass, C3 grass-legume mix, or other forage species.

**Table 4. Fermentation-treatment least squares natural logarithm of DM loss (% of DM) means**

| Fermentation treatment <sup>1</sup> | Least squares mean natural Log DM loss, % of DM | SE    | DM loss least squares mean, exp(Log DM loss) |
|-------------------------------------|---|-------|--|
| Control—untreated                   | 1.49 <sup>a</sup>                               | 0.140 | 4.45   |
| BUCH                                | 1.42 <sup>ab</sup>                              | 0.189 | 4.12   |
| LAB                                 | 1.36 <sup>b</sup>                               | 0.141 | 3.88   |
| AID                                 | 1.18 <sup>b</sup>                               | 0.166 | 3.26   |
| COM                                 | 1.18 <sup>b</sup>                               | 0.172 | 3.24   |

<sup>a,b</sup>Means with similar superscripts do not differ ( $P < 0.05$ ).

<sup>1</sup>Fermentation treatments were categorized into 5 levels: control—untreated, lactic acid-producing bacterial inoculant not containing *Lactobacillus buchneri* (LAB), bacterial inoculant containing *Lactobacillus buchneri* (BUCH), bacterial inoculant containing both LAB and BUCH (COM), or other forage-preservation aid (AID).

capacity. Legumes typically contain greater potassium, calcium, and other cation concentrations than do grasses and other forage species. Pirhofer-Walzl et al. (2011) evaluated forage grasses, legumes and herb mineral contents and found legumes and herbs contained greater cation concentrations relative to pasture grasses. Greater cation concentration will buffer the silo environment and can lead to resistance in pH change during forage acidification (fermentation).

Beyond the interaction discussed previously, forage treatment tended ( $P < 0.102$ ) to be related to fermentation losses. Resulting least squares means for forage-treatment categories are presented in Table 4. Our results agree with prior observations where forage treatments have been described to improve fermentation efficiency relative to untreated controls (Weinberg and Muck, 1996). The forage-preservation-aid treatment improved DM recovery on average relative to control labeled treatment means. This observation agrees with that of Muck (1988), who described that acid application has long been a principle additive type in Europe and acids act to immediately decrease the forage pH, thus improving forage preservation and reducing losses. Bacterial inoculant treatments containing lactic acid-producing bacteria and bacterial inoculant treatments containing lactic acid-producing bacteria combined with *Lactobacillus buchneri* also improved DM recovery, whereas

control demonstrated greater DM losses.

Forages treated with *Lactobacillus buchneri* alone have been shown to increase DM losses (Kleinschmit and Kung, 2006). Yet, our results differed and showed bacterial inoculants containing *Lactobacillus buchneri* similar in DM recovery to both treated and control forages. The final model presented here differed from that of Kleinschmit and Kung (2006) in that both lactic and acetic acid effects were accounted for and separated from inoculant effects in relation to DM recovery. The model difference between reports may explain observed differences.

The results of this meta-analysis, primarily evaluating fermentation outcome, may partly be related to oxygen infiltration. Although oxygen level or penetration was not addressed in this research, limiting oxygen infiltration into silos should also be a management priority in addition to factors investigated here to promote an optimal fermentation. Field strategies such as sealing with concrete, simple plastic (Oelberg et al., 1983), or oxygen-barrier plastic (Borreani et al., 2007) can be implemented to promote an anaerobic environment and limit DM losses. Only after ensuring an anaerobic environment should forage producers focus on the factors evaluated here.

In summary, forage-preservation losses due to fermentation can be substantial. Through our meta-analysis,

fermentation end-product measures, fermentation length, forage species, and preservative parameters were related to forage DM losses following fermentation. Each of the parameter relationships discussed can be considered when evaluating forage-preservation efficiency for opportunities. The aim should be to rapidly decrease forage pH through an adequate, yet not excessive, amount of fermentation acid. Our results suggest forage pH and forage DM content are strongly related to fermentation efficiency. Forage DM less than 30 to 35% at ensiling should be avoided. Implementing strategies to rapidly decrease forage pH, such as ensuring adequate substrate (water-soluble carbohydrate) are available for fermenting bacteria, quickly creating an anaerobic storage environment, and application of lactic acid-producing bacterial inoculants can help mitigate forage DM losses during fermentation. Furthermore, forage species and other forage preservatives such as formic or propionic acid, sodium benzoate, potassium sorbate, and sodium nitrite also affect fermentation losses and should be considered to optimize amount of forage fed relative to that harvested.

## IMPLICATIONS

Forage fermentation end-product parameters in addition to forage DM, forage biology, and fermentation treatment were found related to and capable of describing most DM

loss variation across a wide range of published reports. The final model described here has utility for predicting forage losses during fermentation and may be useful to diagnose problematic fermentations and assess opportunity costs.

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## APPENDIX



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