Prediction models of silage fermentation products on crop composition under strict anaerobic conditions: A meta-analysis

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ABSTRACT

A meta-analysis was conducted to establish linkages between crop and fermentation variables. Data from well-controlled mini silage studies were used in which no additives had been used and no ingress of air had occurred. The silage set consisted of data on crop chemical composition and epiphytic lactic acid bacteria count, and fermentation products (organic acids, alcohols, and ammonia-N) from 118 silages made from 30 grass, 7 legume, 15 grass and legume mixtures, and 66 whole-crop maize samples. The prediction models for fermentation products on crop variables were obtained by stepwise multiple regression analysis. Perennial forage and maize silages were analyzed separately. The best models were obtained for acetic acid in perennial forage silages, with a coefficient of determination of 0.63, and for lactic acid and ethanol in whole-crop maize silages, with coefficients of determination of 0.84 and 0.61, respectively. Fermentation products of perennial forage and maize silages were best related to dry matter and crude protein contents, respectively. Overall, the prediction equations were weak.

Key words: fermentation product, meta-analysis, prediction model, silage

INTRODUCTION

In countries where forage production is limited to a short period during the year, conservation as silage is vital to provide feeds for ruminants throughout the year. Silage production has increasingly become the dominant form of forage conservation in many parts of the world, mainly because of its lower dependency on dry weather compared with hay making.

End products of fermentation largely determine the hygienic and nutritive value of silage, affecting animal performance, milk composition, and milk product qual-

ity. High concentrations of ammonia and organic acids have been shown to decrease silage intake (Huhtanen et al., 2007). Increased levels of lactic acid or total acid also decrease milk fat and protein concentrations (Huhtanen et al., 2003). High levels of ethanol in silages can decrease milk yield but increase milk fat and protein concentrations and also induce milk off-flavor (Randby et al., 1999).

The composition of fermentation products can vary extensively and often unpredictably. Some attempts have been made to predict ensiling results of green silage crops from pre-ensiled composition (Wilkinson et al., 1983; Pitt et al., 1985; Leibensperger and Pitt, 1987). However, these studies either provided approximate estimations of silage quality or did not take into account formation of end-products such as alcohols. To what extent formation of individual end-products relates to initial composition of silage crops remains unclear.

The aim of this study was to obtain prediction models for fermentation products. We hypothesized that crop composition and field flora could explain fermentation product concentrations under strict anaerobic conditions.

MATERIALS AND METHODS

Silage Database

A silage database was constructed from laboratory studies performed at the Department of Animal Nutrition and Management of the Swedish University of Agricultural Sciences (Uppsala) between 1994 and 2011. The database contained 118 observations (30 grasses, 7 legumes, 15 grass and legume mixtures, and 66 whole-crop maize). All crops were grown in Sweden at latitudes of 58 to 60°N. Perennial forages varied in stage of maturity and cut number. Similarly, data on whole-crop maize covered a wide range of maturity stages, including early-harvest observations. Ensiling length and silo type varied from 90 to 151 d and from 1.5-L glass silos to 25-L stainless steel silos. All silos were airtight and kept at 20 ± 2°C during the ensiling

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Table 1. Arithmetical mean, range, and standard deviation (SD) of crop and fermentation variables of perennial forages (grass, legume, and mixture of grass and legume) and whole-crop maize (g/kg of DM unless otherwise stated)

Item ¹	Perennial forages				Maize			
	Mean	Range	SD	No.	Mean	Range	SD	No.
Crop variable								
DM (g/kg)	346	131–623	139.9	52	291	200–406	56.4	65
CP	152	61–220	33.4	52	83	58–103	9.5	66
WSC	119	27–333	58.7	52	108	10–235	59.4	66
Starch	—	—	—	—	208	13–400	113	65
aNDFom	—	—	—	—	447	351–553	56.2	65
Ash	—	—	—	—	45	36–58	5.7	65
BC (g of LA/100 g of DM)	5.6	3.8–10.7	1.4	34	—	—	—	—
LAB (log cfu/g of FM)	3.4	1.2–5.5	1.2	39	4.9	2.5–7.6	1.5	50
Fermentation variable								
Ammonia-N (g/kg of total N)	96.6	0.2–414.5	87.1	50	84.5	56.8–139.7	18.4	66
Lactic acid	34.5	0.1–133	34.7	52	50	11.8–95.2	20.8	66
Acetic acid	13.7	0.3–82.8	16.6	52	18.5	4.3–55.8	11.6	66
Butyric acid	16.4	0.1–105.9	26.5	42	0.7	0.1–11.7	1.9	63
Propionic acid	5.6	0.1–57.5	9.9	36	1.6	0.1–14.3	2.8	63
2,3-Butanediol	8.7	0.1–90	15.9	46	4.2	0.1–18.5	3.6	63
Ethanol	16.6	2.5–131.6	18.7	48	11.5	0.9–34.7	7.6	66

¹WSC = water-soluble carbohydrates; aNDFom = amylase-treated ash-free NDF; BC = buffering capacity; LA = lactic acid; LAB = lactic acid bacteria; FM = fresh matter.

period. No additive treatments were included in the database. Crop variables included DM, water-soluble carbohydrates (**WSC**), buffering capacity (**BC**), CP, amylase-treated ash-free NDF, ash, starch, and lactic acid bacteria (**LAB**). Silage composition included organic acids (lactic, acetic, propionic, and butyric acids), alcohols (ethanol and 2,3-butanediol), and ammonia-N. Arithmetical mean, range, and standard deviation of crop and fermentation variables are given in Table 1.

Laboratory Analyses

Crop samples were dried at 60°C overnight and milled to pass a 1-mm sieve before being subjected to chemical analyses. The DM concentration was measured after drying at 103°C and before ashing at 550°C for 3 h. Concentration of CP was estimated by multiplying N concentration measured by the Kjeldahl technique to 6.25. Starch and WSC were measured by using an enzymatic method described by Udén (2006, 2010). The method described by Van Soest et al. (1991) and modified by Chai and Udén (1998) was used for determination of amylase-treated ash-free NDF. Crop BC was measured according to McDonald and Henderson (1962). Culture-dependent microbial analysis was used to quantify LAB with Rogosa agar as the culturing medium (Seale et al., 1986).

Organic acids and alcohols were measured on extracted silage juice by HPLC (Andersson and Hedlund, 1983). The method described by Broderick and Kang (1980) was used to measure ammonia-N.

Statistical Analyses

All statistical analyses were carried out using SAS software (version 9.2; SAS Institute Inc., Cary, NC). Relationships within crop and fermentation variables were studied by correlation analysis, and significance was declared at $P < 0.05$. The prediction models for fermentation products were obtained using the REG procedure, in which multiple regression models were fitted by the STEPWISE model-selection method. Variables were entered and retained in the model at $P < 0.15$. The Univariate procedure was used to test the normality of residuals, and logarithmic transformation of response variables was applied if not normally distributed. Perennial forage and maize silages were analyzed separately. The effect of study was not included in the statistical model because the data set included only one observation per study.

RESULTS

Correlation Analysis of Fermentation Products

Pearson correlation coefficients between fermentation products of perennial forage and whole-crop maize silages are presented in Tables 2 and 3, respectively. In both silage categories, ammonia-N was positively correlated with butyric acid and 2,3-butanediol. Several discrepancies, however, were found between perennial forage and maize silages regarding correlation results. For instance, ammonia-N and acetic acid were positively correlated in perennial forage silages but uncor-

Table 2. Pearson correlation coefficients between fermentation products of perennial forage (grass, legume, and mixture of grass and legume) silages

	Ammonia-N	Lactic acid	Acetic acid	Butyric acid	Propionic acid	2,3-Butanediol	Ethanol
Ammonia-N	—						
Lactic acid	NS	—					
Acetic acid	0.771***	NS	—				
Butyric acid	0.774***	−0.365*	0.714***	—			
Propionic acid	NS	NS	0.421*	NS	—		
2,3-Butanediol	0.453**	NS	NS	NS	NS	—	
Ethanol	NS	NS	NS	NS	NS	NS	—

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = $P \geq 0.05$.

related in maize silages. Interestingly, ethanol was not associated with any end-products in perennial forage silages but positively correlated with lactic acid and acetic acid in maize silages.

Regression Analysis

Perennial Forage Silages. Regressions of fermentation products on crop variables of perennial forage silages are presented in Table 4. Increasing DM concentration negatively affected the concentration of all fermentation products. This effect was, however, markedly greater for ammonia-N, acetic acid, butyric acid, and propionic acid, as DM accounted for more than 74% of the total explained variation by the fitted models. The equations for ammonia-N and butyric acid included similar regressors (DM, WSC) with similar weights in the respective models. The best model was obtained for acetic acid with a R^2 value of 0.63 and with DM, LAB, and CP being included. The residuals of the models for lactic acid, propionic acid, 2,3-butanediol, and ethanol were not normally distributed and values were therefore log-transformed. However, this problem was not reduced for the lactic acid model and is therefore not reported.

Maize Silages. Regressions of fermentation products on crop variables of maize silages are shown in Table 5. The CP concentration showed a strong association with lactic acid, ethanol, and acetic acid, accounting for more than 79% of the total explained

variation by their respective models. Starch had a similar role in models of ammonia-N and butyric and propionic acids, in which more than 67% of the total explained variation was due to this variable. In contrast to the perennial forage silages, in maize silages DM explained a negligible proportion of end-product variation and only for the 2 alcohols. The best regressions were obtained for lactic acid and ethanol, with crop composition explaining 0.84 and 0.61 of the variation, respectively.

DISCUSSION

Correlation Analysis

The formation proportion of end-products is affected by several factors, including dominant microorganisms, fermented substrates, and the fermentation pathways undertaken by the individual groups of silage microorganisms. The latter factor is determined by environmental factors such as pH, substrate availability, and reduction-oxidation status of the system (McDonald et al., 1991). It appears that the correlation type between end-products is a function of several factors; hence, a fixed correlation may not always exist. As an example, lactic and acetic acids can be formed in a 1:1 ratio in heterolactic fermentation of glucose by LAB. They may also be derived from independent fermentation pathways when multiple substrates and microorganisms are involved (McDonald et al., 1991). In the first scenario,

Table 3. Pearson correlation coefficients between fermentation products of whole-crop maize silages

	Ammonia-N	Lactic acid	Acetic acid	Butyric acid	Propionic acid	2,3-Butanediol	Ethanol
Ammonia-N	—						
Lactic acid	NS	—					
Acetic acid	NS	0.443***	—				
Butyric acid	0.645***	NS	NS	—			
Propionic acid	0.646***	NS	NS	0.794***	—		
2,3-Butanediol	0.718***	−0.370**	NS	0.687***	0.705***	—	
Ethanol	NS	0.627***	0.477***	NS	NS	NS	—

** $P < 0.01$; *** $P < 0.001$; NS = $P \geq 0.05$.

Table 4. Stepwise regression models of fermentation products on crop variables of perennial forage (grass, legume, and mixture of grass and legume) silages (g/kg of DM unless otherwise stated)

Item ¹	Intercept/slope	SE	P-value	R ² cumulative
Ammonia-N (g/kg of total N)				
Intercept	295.68	42.02	<0.001	
DM (g/kg)	-0.45	0.12	<0.01	0.34
WSC	-0.42	0.21	0.06	0.43
Acetic acid				
Intercept	8.52	15.50	0.59	
DM (g/kg)	-0.10	0.02	<0.0001	0.47
LAB (log cfu/g of FM)	4.89	2.00	0.02	0.58
CP	0.13	0.07	0.08	0.63
Butyric acid				
Intercept	77.92	12.22	<0.0001	
DM (g/kg)	-0.15	0.04	<0.001	0.41
WSC	-0.12	0.06	0.06	0.48
Propionic acid				
Intercept	1.28	0.27	<0.0001	
DM	-0.003	0.001	<0.01	0.30
2,3-Butanediol				
Intercept	2.24	0.56	<0.001	
DM	-0.003	0.001	0.01	0.14
LAB (log cfu/g of FM)	-0.27	0.11	0.02	0.29
Ethanol				
Intercept	1.09	0.14	<0.0001	
WSC	0.003	0.001	<0.001	0.29
DM	-0.001	0.0004	0.01	0.46

¹WSC = water-soluble carbohydrates; LAB = lactic acid bacteria; FM = fresh matter.**Table 5.** Stepwise regression models of fermentation products on crop variables of whole-crop maize silages (g/kg DM unless otherwise stated)

Item ¹	Intercept/slope	SE	P-value	R ² cumulative
Ammonia-N (g/kg of total N)				
Intercept	60.43	4.12	<0.0001	
Starch	0.12	0.02	<0.0001	0.43
Lactic acid				
Intercept	-87.41	18.98	<0.0001	
CP	1.15	0.23	<0.0001	0.67
Ash	2.18	0.47	<0.0001	0.78
NDF	-0.20	0.05	<0.001	0.81
LAB (log cfu/g of FM)	4.99	1.81	0.01	0.83
WSC	0.06	0.03	0.07	0.84
Acetic acid				
Intercept	-0.42	0.27	0.12	
CP	0.02	0.003	<0.0001	0.45
Butyric acid				
Intercept	-3.31	0.75	<0.0001	
Starch	0.004	0.001	<0.0001	0.25
Ash	0.04	0.01	0.01	0.37
Propionic acid				
Intercept	-3.29	0.69	<0.0001	
Starch	0.004	0.001	<0.0001	0.31
CP	0.02	0.01	<0.01	0.44
2,3-Butanediol				
Intercept	4.35	1.07	<0.001	
WSC	-0.01	0.002	<0.0001	0.34
CP	-0.02	0.01	<0.01	0.42
DM (g/kg)	-0.004	0.002	0.05	0.47
Ethanol				
Intercept	-2.49	0.46	<0.0001	
CP	0.03	0.004	<0.0001	0.52
DM (g/kg)	0.003	0.001	<0.01	0.61

¹LAB = lactic acid bacteria; FM = fresh matter; WSC = water-soluble carbohydrates.

lactic acid and acetic acid would be positively correlated, whereas in the latter case, a positive correlation is not obvious.

The inconsistent correlation results for end-products between perennial forage and maize silages suggest that fermentation type differed between them. For perennial forage silages, the fermentation results (Table 1) suggest an overall dominance of clostridial fermentation. This was most likely due to the inherent tendency of perennial forages for this type of fermentation. In addition, the low initial counts of LAB (Table 1) could have played a role. On the other hand, for maize silages, the fermentation profile (Table 1) suggests that clostridial activity was low (i.e., low butyric acid concentration) and fermentation was dominated by LAB and enterobacteria. In maize silages, clostridia do not usually dominate fermentation, particularly under strict anoxic conditions, because the rapid pH reduction prevents their growth. The relatively high level of ammonia-N was therefore likely the result of enterobacterial fermentation.

Regression Analysis

In regression analysis, intercorrelation among explanatory variables causes inflation in standard errors of the estimated parameters, known as collinearity (Mason, 1987). This will severely reduce their robustness. In the maize silage database, crop variables had high inter-correlations (results not shown). The variance inflation factors test in the REG procedure suggested, in some cases, considerable collinearity. However, as the objective of this study was to find useful end-product prediction models, not robust estimates of individual parameters, existence of collinearity was ignored.

During ensiling, DM, WSC, and BC are known to be the most important crop chemical variables affecting the result, which was evident in the regressions obtained for the perennial forage silages. A significant effect of DM was observed in all the models and a contribution of WSC was also present (Table 4). The absence of a BC effect could be a result of the large number of missing values for this variable in the data collected (Table 1). For maize silages, on the other hand, the DM range was narrow (Table 1) and, therefore, contribution of DM in fermentation models was limited, as expected. The marked contribution of CP in the prediction models of lactic acid, acetic acid, and ethanol could have been indirect, in part, and an indication of the occurrence of heterolactic fermentation, because we observed a positive correlation between CP and LAB counts ($r = 0.54$, $P < 0.001$). The contribution of starch as a substrate is minor, if any; hence, the effect of starch here could be seen from its positive correlation with DM ($r = 0.81$,

$P < 0.0001$) and inverse correlation with WSC ($r = -0.77$, $P < 0.0001$).

Data collected in this study came from well-controlled laboratory silage studies to ensure uniform ensiling conditions (e.g., temperature, air penetration). However, the obtained prediction models for fermentation products were relatively weak (Tables 4 and 5). This implies that crop chemical composition, BC, and LAB counts may be insufficient to explain variation in fermentation products. Using a similar approach but including only DM, WSC, and BC as the prediction variables, Wilkinson et al. (1983) also obtained poor prediction models for fermentation results. One of the extant shortcomings in silage science is the lack of an appropriate measure for fermentable substrates that become available during the course of fermentation. This fraction may originate from structural carbohydrates (e.g., hemicelluloses and pectic substances) and can be released by acid hydrolysis, enzymatic breakdown, or both (Pitt et al., 1985; Muck, 1988; McDonald et al., 1991). One possible way to estimate this fraction is to calculate carbon balance between fermentation products and pre-ensiled contents of known fermentable substrates, including WSC, amino acids, and organic acids.

For a successful ensiling in which LAB dominate fermentation, the prerequisites are rapid achievement of anaerobic condition and its maintenance, sufficient amount of fermentable substrates, and a minimum number of 10^6 LAB per gram of pre-ensiled crop (Muck, 1988; Muck and Pitt, 1993). The latter criterion is not met in most circumstances (Muck and Pitt, 1993), which is also apparent in the collected data here. In perennial forage silages, the LAB count was below the desirable level for all observations, and in maize silages, the desirable level was only met for 30% of the observations (Table 1). This suggests that low initial LAB contents provided opportunities for other competing microbes to contribute significantly to the fermentation results observed. Hence, elaborate data on initial microbial composition could have substantially enhanced the predictive power of the regression models.

Detailed information on available and potentially available fermentable substrates and composition of the field flora are required to enable better predictions. It also seems likely that water activity should be more informative than DM content because it has a direct influence on the activity of microorganisms (Madigan et al., 2012).

CONCLUSIONS

Prediction models based on available chemical and microbial crop variables had limited explanatory value. The best equations were obtained for acetic acid in

perennial forage silages and for lactic acid and ethanol in whole-crop maize silages with R^2 values of 0.63, 0.84, and 0.61, respectively. The DM and CP concentrations of perennial forages and maize crops, respectively, had the strongest effects on fermentation products.

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