VFA PRODUCTION IN THE RUMEN OF SHEEP FED LIMESTONE AND UREA TREATED CORN SILAGES

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Previous research clearly showed that the addition of limestone to corn plant material at ensiling time increased the production of organic acids in corn silage (Klosterman et al., 1961; Byers, Davis and Baylor, 1964; Simkins, Baumgardt and Niedermeier, 1965 and Johnson and McClure, 1968). Klosterman et al. (1961) reported increased feed efficiency in eight trials when limestone treated corn silage was fed to beef steers and heifers as compared to untreated corn silage. In four of the trials, the cattle fed limestone-treated silage made faster gains. It was suggested that the increased gains and efficiency might be associated with the increased organic acid content of the silages. In feeding trials with lambs (Bentley, Klosterman and Engle, 1955) beef cattle (Johnson, Klosterman and Bentley, 1962) and dairy heifers (Emery et al., 1961), lactic acid appeared to have a high feed replacement value as an energy source. The present study was conducted to investigate the effects of limestone and urea treatments of corn silage on the volatile fatty acid (VFA) production in the rumens of sheep consuming these silages.

Materials and Methods

Silage Preparation. Batches of approximately 600 kg. of chopped corn plant material harvested in the glaze stage of ear development were spread on a smooth floor. The specified additives (table 1) were sprinkled over the top of the material and it was thoroughly mixed with a shovel. This material was ensiled in 80 kg. capacity black polyethylene bags (Johnson et al., 1966) until fed in the in vivo study.

Feeding and Sampling Trial. Four 35 kg. rumen fistulated wether lambs were used in a 4 x 4 latin square design trial using the silages shown in table 1. Prior to the start of the experiment the sheep were fed corn silage for a 30-day adjustment period during which they were allowed to eat ad libitum. Four days prior to the start of the experimental trials their intakes were adjusted to 85% of that consumed by the sheep with the lowest voluntary intake and they were trained to consume this in a short period of time. Thus each sheep was fed 2.7 kg. of fresh silage divided into two equal feedings at 7:00 a.m. and 7:00 p.m., both of which were usually consumed 1/3 hr. after feeding.

Each period of the study consisted of a 10-day adjustment period and a 6-day collection period. Samples of rumen fluid were obtained at prefeeding and at 1, 2, 4, 8 and 12 hr. postfeeding on days 2, 4 and 6 of the collection period. Approximately 200 ml. of rumen fluid were obtained at each sampling by means of a sampling tube inserted through the fistula and pH was determined immediately after removal. After determining pH, the rumen fluid was strained through two layers of cheesecloth and placed in 100 ml. plastic bottles. These were frozen quickly by insertion into a liquid nitrogen tank and stored in the deep freeze. For VFA analysis, aliquots of the thawed fluid were mixed 4:1 with 25% metaphosphoric acid and centrifuged. The supernatant was injected directly into a gas chromatograph equipped with a flame ionization detector. Peak areas on the recorder chart were compared to standards run on each day of analysis. Acetic acid was determined on the silage extracts by the same procedure. Lactic acid was determined on the gas chromatograph according to the procedure of Hoffman, Barboriak and Hardman (1964).

Analysis of variance was conducted by the method of least squares (Harvey, 1960).

Results and Discussion

The pH and organic acid content of the silages used are shown in table 1. As has been
previously reported, limestone treatment markedly increased the lactic acid content of the silage but had a lesser effect on the acetic acid content. Urea treatment alone had no effect on acid concentrations and final pH of the silage was slightly higher in limestone treated silages but unaffected by urea treatment.

Figure 1 shows the pH and the concentration of total volatile fatty acids (TVFA) in the rumen fluid when the four silages were fed. The pH generally decreased after feeding in all cases from a prefeeding value of

![Figure 1. Rumen pH and total volatile fatty acids (TVFA) at intervals after feeding control and limestone or urea treated corn silages. Points on the curves with different letter designations at a given time period are significantly different (P<.05).](image-url)
7.5 to around 6.9 to 7.0 1 hr. after feeding and did not start to increase again until after 4 hr. postfeeding. No differences between silages appeared except 2 hr. postfeeding at which time the rumen pH for the animals fed urea treated silage was significantly ($P < .05$) lower than the other treatments. This observation cannot be explained at this time.

TVFA concentrations increased rapidly during the first hour postfeeding and only slightly more to a maximum at 2 hr., after which they generally declined. At both 1 and 2 hr. postfeeding, TVFA concentrations were significantly ($P < .05$) higher in rumen fluid from animals fed treated silages than from those fed the control silage. These differences persisted up to the 4th hr. with the limestone treated silages. Thus, the data suggest that rumen pH was not directly associated with differences in TVFA.

Figures 2, 3 and 4 present the proportions

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**Figure 2.** Acetic acid expressed as mg. per 100 ml. and as molar % in rumen fluid after feeding control and limestone or urea treated corn silages. Points on the curves with different letter designations at a given time period are significantly different ($P < .05$).
of acetic, propionic and butyric acids in the rumen fluid in terms of both molar percentage and concentration in milligrams per 100 milliliters. Quite logically, the concentration of all three acids increased after feeding since all would be synthesized during the active metabolic period. Increases in acid levels were definitely not proportional for the three acids, however. The molar percent of acetic acid dropped rapidly from around 64% at feeding to 54 to 57% at 2 hr. postfeeding after which it returned slowly to prefeeding levels. Conversely, the molar % of propionic acid increased sharply during the first 2 hours. Molar % butyric acid increased considerably with the control ration but somewhat less with the others. Thus, with all the silage rations, the fermentation during the first few hours postfeeding was predominantly a propionic acid producing one. These results agree with those reported by Putnam, Yarns and Davis (1966).
The concentration of acetic acid was significantly (P < .05) higher at 1 and 2 hr. postfeeding in the lambs receiving treated silage compared to the control silage. This was generally true for propionic acid also. In the case of propionic acid, however, the concentration was significantly (P < .05) higher in the limestone and urea-limestone silage fed animals than in those fed the urea treated silage. Furthermore, this difference persisted through 4 hr. postfeeding. In contrast, butyric acid was highest in the animals fed control silage at 2 hr. postfeeding but only significantly so in comparison with the animals fed limestone treated silage. Other differences noted with butyric acid could not be consistently associated with either limestone or urea treatment.
Similar data for isovaleric and n-valeric acids are shown in figures 5 and 6. Although there was an increase in isovaleric acid following feeding, it peaked at 2 hr. and decreased rapidly thereafter. Molar % isovaleric generally decreased until 4 hr. postfeeding. No consistent differences could be associated with rations. N-valeric acid concentration increased slowly the first hour postfeeding and more rapidly the second hour, after which it decreased slowly. Molar % n-valeric acid also increased between the first and second hour. Although the differences were not always significant, there was a consistent trend for n-valeric acid to be lower in the animals fed the urea treated silage. The metabolic significance of this effect is not apparent.

The increase in TVFA due to silage treatment were accounted for almost entirely by increases in acetic and propionic acids. Pro-

![Diagram](image)

**Figure 5.** Isovaleric acid expressed as mg. per 100 ml. and as molar % in rumen fluid after feeding control and limestone or urea treated corn silages. Points on the curves with different letter designations at a given time period are significantly different (P<.05).
portionally, propionic acid was increased more since its molar % increased while that of acetic decreased. Considering this and the fact that the increases were in the first two hours it can be assumed that the effects can be associated with fermentations of starch, soluble carbohydrates or organic acids in the silage. Johnson et al. (1966) found that 40 to 80% of the soluble carbohydrates in corn plant material were fermented in the ensiling process. Prolonging the fermentation by urea-limestone additions would undoubtedly increase those percentages and the treated silages would have less residual soluble carbohydrate for rumen fermentation than untreated silage. Thus, an increased fermentation to VFA from the treated silages would be more likely to be associated with either starch fermentation or metabolism of the higher lactic acid content. Walker (1968),

![Graph showing valeric acid levels in rumen fluid after feeding control and limestone or urea treated corn silages.](image)

Figure 6. Valeric acid expressed as mg. per 100 ml. and as molar % in rumen fluid after feeding control and limestone or urea treated corn silages. Points on the curves with different letter designations at a given time period are significantly different (P<.05).
in a review of the role of lactic acid in ruminant nutrition, points out that the formation of lactic acid \textit{in vivo} or the addition of it in the ruminant diet is invariably associated with higher propionic acid proportions in the rumen VFA’s. On the other hand, \textit{in vitro} and isotope studies show that lactate can be converted to acetic, propionic or butyric acids in varying proportions, sometimes with acetic acid being the predominant end product. The rapid disproportionate increase in propionic acid (1 hr.) when the high lactate silages were fed in this study suggest that lactate was being converted predominantly to propionate.

On the other hand, a similarly higher TVFA was demonstrated in the rumen fluid of animals fed the urea treated silage which did not have an elevated lactic acid content. In this case, however, the proportion of acetic acid was significantly higher than with the other silages (figure 2), suggesting a different alteration in speed or type of fermentation. An increased rate or change in type of fermentation could possibly be associated with the presence of readily available NPN.

It would appear then that fermentation patterns in the rumen are changed when limestone or urea treated corn silage is consumed in comparison to untreated silage. Whether these changes are due to the presence of additional Ca$^{++}$ ions, NH$_4^+$ ions (as a N-source) or lactic acid, any of which might be acting independently or together, remains to be determined. What is equally interesting is speculation on the relationship of these changes to the increased efficiency of utilization of limestone or urea treated corn silages by beef cattle. Some possible explanation might come from recent studies on milk fat depressing rations with dairy cows. Generally, the situation of depression of milk fat is associated with increased propionate production in the rumen and a decreased acetate/propionate ratio (Van Soest, 1963). The reports of Opstvedt, Baldwin and Ronning (1967), Opstvedt and Ronning (1967) and Baldwin \textit{et al.} (1969) show that under conditions of high concentrate feeding (and resultant high propionate production in the rumen) the activities of enzymes associated with fatty acid synthesis and esterification in adipose tissue are increased several fold. In addition, the quantity of \textit{\alpha}-glycerol phosphate, a requirement for triglyceride synthesis in adipose tissue, is increased under these circumstances. Since propionate is a potential precursor of $\alpha$-glycerol phosphate it is tempting to postulate that shifts toward propionate production such as discussed in this paper might bring about more efficient utilization of energy by stimulating more rapid deposition of adipose tissue. Obviously, proof of this postulation for fattening beef cattle awaits further research.

**Summary**

Well eared corn plant material was ensiled alone or with 0.5% limestone, 0.5% urea or 0.5% limestone+0.5% urea and fed to fistulated lambs. Limestone treatment increased lactic acid content in the corn silage but had no effect on acetic acid or pH.

Rumen pH decreased from 7.5 prefeeding to 6.9 1 hr. postfeeding after which it slowly increased to prefeeding levels. Silage treatment had no consistent effect on rumen pH. Total VFA in the rumen increased rapidly during the first hour postfeeding and to a significantly higher level with the treated silages. TVFA levels reached a maximum at 2 hr. postfeeding and declined thereafter.

Although the levels of all VFA’s increased after feeding, the molar \% of propionic acid increased while that of acetic acid decreased up to 2 hr. postfeeding. During the same period, molar \% butyric increased somewhat (control silage) or remained the same and molar \% isovaleric decreased up to 4 hr. post-feeding. The proportion of propionic acid was significantly higher after feeding limestone treated silages than after feeding the other silages. A significantly higher proportion of acetic acid was observed at 1 hr. after feeding the urea treated silage as compared to all other silages. Molar proportions of butyric acid were highest after feeding the untreated silage. The possible relationship of these fermentation patterns to increased Ca$^{++}$ and NH$_4^+$ ions and lactic acid are discussed.

**Literature Cited**


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