Ammonia-N and α-amino-N in silage determined on either water extracts or solubilized freeze-dried samples

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Introduction Drying of silage samples causes losses of volatiles, of which ammonia is reported to disappear completely by oven drying at 60° C (Porter and Murray, 2001) as well as partially by drying at 80° C (Sorensen 2004). Freeze-drying is commonly not assumed to cause losses of ammonia, although freeze-drying studies on silage generally report total weight losses rather than ammonia losses. Fecal samples are another sample category where ammonia losses would be likely to occur. Spanghero and Kowalski (1997) reported that 12% of total N content disappeared by freeze-drying dairy cow fecal samples. In research work it is usual that ammonia N is analyzed together with α-amino-N, which, although not being a volatile fraction, still could undergo changes during freeze-drying. The objective of this study was to assess the changes in concentration of ammonia N and α-amino-N when analyzed in water extracts obtained either from fresh material or from freeze-dried samples of whole silage.

Material and Methods One batch from the primary growth from each of timothy grass (22.0 g N/kg DM) and red clover (33.2 g N/kg DM), respectively, was subjected to ten different combinations of wilting length (0-52 h) and target dry matter (130-750 g/kg) before ensiling in triplicates for 115 d in 4.5 L PVC silos. This created a variation in fermentation profiles, with the sum of acids and alcohols ranging from 11 – 221 g/kg DM, pH 4.38 – 5.50 and with NH_3-N constituting 14 – 207 g/kg N.

After silo opening, the samples were prepared for determination of solubles by the routine procedure of our lab. This comprises weighing 100 g fresh material into ziplock bags, addition of equal weights of deionized water, freezing, thawing and hydraulic pressing of juice from the bag after puncturing it. Parallel samples of whole silage were freeze-dried in a CD 8 freeze-drier (HETO, Birkerød, Denmark) and milled on a hammer mill to pass a 1 mm screen whereafter 1.2 g sample was weighed into a 50 mL conical plastic centrifuge tube with 40 mL of deionized water. The tubes were agitated for 60 min on a tube shaker/rotator with 360° vertical rotation at 21 rpm, centrifuged for 5 min at 1800 × g on a swing-out centrifuge and the supernatant was decanted to 7 mL tubes, subsequently transferred to Eppendorf tubes and centrifuged for 5 min at 13000 × g. The solubilized samples, as well as centrifuged juice samples from hydraulic pressing, were then diluted with deionized water at ratios 1:4 and 1:19 (v/v), respectively. Both sample types were analyzed for NH_3-N and α-amino-N on a Technicon AutoAnalyzer with phenol-hypoclorite and ninhydrin as main reagents (Broderick and Kang 1980). The results were recalculated to a per kg DM basis after DM determination at 60°C on the milled samples without employing any DM correction for losses of volatiles.

Result Average sample contents of NH_3-N were 2.96 g/kg DM (range: 0.32 – 7.88 g/kg DM) determined on pressed juice and 2.99 g/kg DM (range: 0.50 – 7.82 g/kg DM) from analysis on solubilized freeze-dried samples. Corresponding results for α-amino-N were 7.26 g/kg DM (range: 1.21 – 11.99 g/kg DM) determined on pressed juice and 8.32 g/kg DM (range: 2.16 – 12.66 g/kg DM) on solubilized freeze-dried samples. Regressions of pressed juice results (y) against solubilized freeze-dried sample results (x) on all 60 samples are presented in Figure 1. Slopes were different from 1 ($p < 0.001$ and $p < 0.05$ for NH_3-N and α-amino-N, respectively). The largest difference between methods for a treatment mean ($n = 3$) was 0.55 g kg/DM lower NH_3-N for freeze-dried samples than for pressed juice, corresponding to 7% of total NH_3-N content in the treatment. For α-amino-N, the largest difference was 1.69 g/kg DM less in pressed juice than in freeze-dried samples, equal to 25% of total α-amino-N in the treatment.

Discussion The moderately positive slope implies that ammonia N disappeared to a proportionally larger extent when concentration was high. However, this was not very pronounced. The absence of a treshold effect, with more or less complete disappearance above a certain ammonia concentration, may be due to that extensive acid production usually accompanies ammonia formation. This means that more anions for salt formation also would be available when ammonia levels increase.

The larger N losses (and hence ammonia losses) found from freeze-drying fecal samples (Spanghero and Kowalski 1997) are probably explained by the compared to silage much higher pH of fecal samples, typically >7 for high yielding cows (Mgbeahurike 2007). Since each pH unit equals a

factor 10, equilibrium in a silage sample would favour the ionized and non-volatile ammonium form by about 10^3 compared to a fecal sample.

There was no obvious explanation for the apparent increase in α-amino-N values after freeze-drying. A possibility is that oligopeptides during freeze-drying to some extent are cleaved to free amino acids and shorter peptides that will give response in the ninhydrin assay. There is also a larger degree of uncertainty involved in α-amino-N determination than in NH_3-N determination with the current method, because the result is obtained after deduction of the ninhydrin response to ammonia.

Conclusion The results suggest that only minor losses of ammonia occur during freeze-drying of timothy/red clover silage with the characteristics described here. The α-amino-N values increased by freeze-drying.

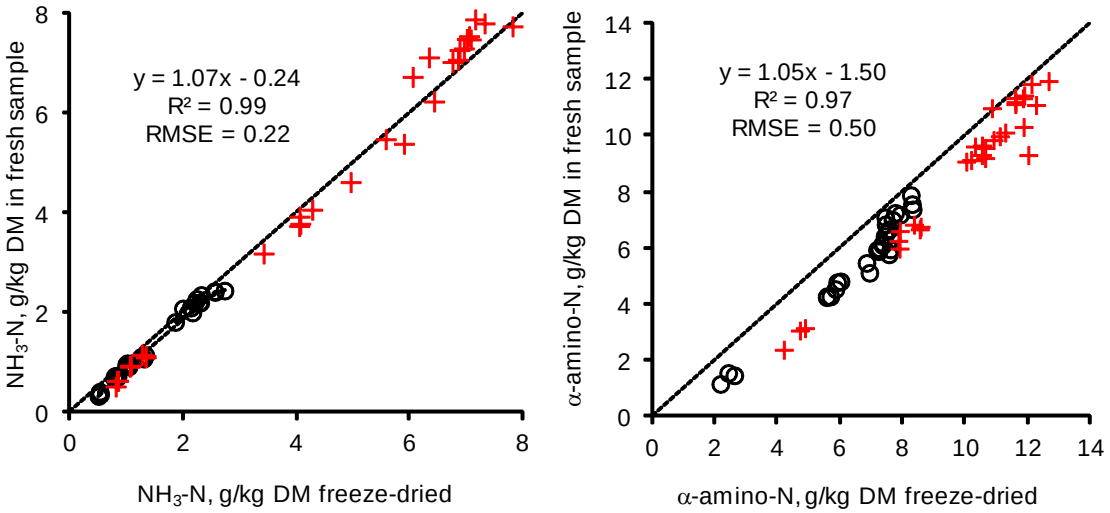

Figure 1 Concentrations of silage N fractions determined in water extracts obtained either from fresh samples or from freeze-dried and milled samples of timothy (**O**) or red clover (**+**). Dashed line represents y = x. RMSE = Root Mean Square Error. N = 60.

References

Broderick, G.A., Kang, J.H. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *Journal of Dairy Science* 63: 64-75.

Mgbeahurike, A. 2007. Faecal characteristics and production of dairy cows in early Lactation. MSc Thesis No. 62. Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Skara. 63 p.

Porter, M.G., Murray, R.S. 2001. The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods. *Grass and Forage Science* 56: 405-411.

Spanghero, M., Kowalski, Z.M. 1997. Critical analysis of N balance experiments with lactating cows. *Livestock Production Science 52*: 113-122.

Sørensen, L.K. 2004. Prediction of Fermentation Parameters in Grass and Corn Silage by Near Infrared Spectroscopy. *Journal of Dairy Science* 87: 3826-3835.