Nitrogen Distribution and Acid Production in Corn Silage Treated with Ammonium Hydroxide

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Treatment of chopped corn plants with ammonium hydroxide to supply nitrogen at levels of 0.00, 0.31, 0.62, 0.93, and 1.24% of dry matter gave a constant increase in nitrogen content which was nearly equivalent to the dose and which was largely represented as ammonium nitrogen. Nonammonium nitrogen represented only a small part of the increase although the proportion of the added nitrogen in this form was generally increased in response to an increase in nitrogen addition. The ammonium nitrogen of the increase was both highly soluble in a slightly acidic citrate buffer extract and quantitatively similar at pre- and postfermentation, and that as nonammonium nitrogen was also soluble as shown at prefermentation. Other effects of an increase in nitrogen addition were a reduction in proteolysis of ensiling, an increase in pH, and an increase in lactic acid content at postfermentation, with the response in lactic acid content being largest with an addition at 0.62 or 0.93%.

The treatment of corn silage with nitrogen as ammonium hydroxide before ensiling has been reported to be effective in increasing the feeding value (Henderson and Bergen, 1972; Huber and Santana, 1972). In studying the chemical composition of the treated silage, Huber et al. (1979) observed that the nitrogen was mostly present as ammonium nitrogen, as much as 47% of which was water insoluble. The present work with corn silage of different batches and preparations also concerned chemical measurements on samples which received nitrogen addition as ammonium hydroxide.

EXPERIMENTAL PROCEDURES

At weekly intervals, three batches of yellow dent corn plants were chopped to an approximate 1-cm particle size and used for treatment with different levels of nitrogen as ammonium hydroxide (23.8% NH_3-N). A farm-type forage chopper was used to harvest the plants which were at the soft dough stage and contained 30% dry matter. Each batch was prepared as material which was undried (30% dry matter), was partly dried (41–42% dry matter), and was partly dried plus a subsequent water addition (30% dry matter). The partly dried material was prepared by air-drying for 6 h at about 25 °C. The nitrogen additions were at 0.00, 0.31, 0.62, 0.93, and 1.24% of the plant dry matter with each material. Each addition was with a single sample. Applications of the ammonium hydroxide reagent plus small amounts of distilled water were hand sprayed onto weighed samples (0.9–1.1 kg of dry matter each). The samples were enclosed by large plastic bags during the treatment and during a 12–16-h equilibration at 2 °C. Air was not excluded, and a small opening was necessary in each bag during the applications. After equilibration, the contents were thoroughly mixed by hand and sampled. The sample portions were stored at −20 °C until analysis. The remaining portions from one of the batches (batch 1) were used for ensiling.

The period of ensiling was 45 days at 28–29 °C. During this period, each sample was kept in a gallon-size polyethylene bottle which had the lid wrapped with vinyl tape plus Parafilm. The contents (0.7–0.8 kg of dry matter each) were tightly packed to fill the container. At completion of the ensiling, the contents were removed, mixed, and sampled for analysis. Also, the wet contents were weighed both before and after the ensiling to enable calculation of analytical results in relation to the plant matter at prefermentation. For treatment involving nitrogen additions of 0.00, 0.31, 0.62, 0.93, and 1.24% to undried material, postfermentation weight recoveries based on original weight were 99.3, 99.1, 99.5, 99.6, and 99.5%, respectively. The same additions to the partly dried material gave values of 98.7, 98.5, 99.0, 99.6, and 99.4%, respectively, and to the partly dried plus water material, the additions gave values of 99.2, 99.2, 99.2, 99.5, and 99.4%, respectively.

Approximately 100 g of each sample was ground to a 1.1-mm average particle size with a Waring blender under cold conditions in preparation for analysis. Each was analyzed in duplicate for total nitrogen (organic plus ammoniacal) by the Gunning method (Association of Official Agricultural Chemists, 1950) and for ammoniacal nitrogen by the magnesium oxide method (Association of Official Agricultural Chemists, 1950). The nonammonium nitrogen was calculated by difference. Other analyses which were made with batch 1 samples included pH (Barnett, 1954), lactic acid [Barker and Summerson (1941), as modified by Pennington and Sutherland (1956)], and acetic, propionic, and butyric acids by gas chromatography. In the chromatographic separation, a 6 ft × $^1/_8$ in. glass column packed with 20% neopentylglycol succinate–2% H_3PO_4 on 60–80-mesh firebrick was used. Samples that were not finely ground were used for all pH measurements and for dry matter determinations on undried and partly dried materials prior to treatment. The dry matter was determined by drying 24 h at 75 °C in a forced draft oven.

Solubility of the total and ammonium nitrogen of the batch 1 samples was studied by means of a slightly acidic extract which would prevent the escape of free ammonia. The extraction was as follows. A finely ground, weighed, wet portion of each sample, which was equivalent to 15.0 g of plant dry matter, was soaked with 100 mL of pH 5.28 citrate buffer (contents per liter in grams: citric acid hydrate 24.55; NaOH of 97% purity, 14.4; concentrated HCl, 6.8 mL) plus 100–115 mL of distilled water and 1 or 2 drops of toluene for 16–18 h at 2 °C. After adjustment of the volume to 250 mL (note that the water displacement of 15.0 g of the silage dry matter averaged 10.7 mL and, therefore, the extract volume was 239.3 mL), the mixture was transferred into a Waring blender along with 10 mL of distilled water as the rinse. The mixture was then ground for 2–3 min and allowed to stand for 30 min at 2 °C. This procedure was repeated once. After the extract was strained through cheesecloth and centrifuged at 6900*g* for 30 min at 2 °C, duplicate aliquots of the extract were analyzed for total and ammoniacal nitrogen by the procedures previously cited. Soluble nonammonium nitrogen

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Table I. Total, Ammonium, and Nonammonium Nitrogen of Chopped Corn Plants of Different Batches and Preparations Which Received Added Nitrogen as Ammonium Hydroxide

added nitrogen, %[a]	nitrogen of different fractions, %[a]		
	total nitrogen[b]	ammonium nitrogen[b]	nonammonium nitrogen (by difference)
0.00	1.127 ± 0.020[c]	0.026 ± 0.003[c]	1.100 ± 0.021[c]
0.31	1.423 ± 0.013	0.312 ± 0.004	1.112 ± 0.014
0.62	1.694 ± 0.024	0.578 ± 0.006	1.116 ± 0.021
0.93	1.982 ± 0.013	0.808 ± 0.011	1.176 ± 0.010
1.24	2.286 ± 0.023	1.040 ± 0.009	1.246 ± 0.017

[a] In reference to the plant dry weight. [b] Nine samples were involved in the total or ammonium nitrogen measurements at each level of nitrogen addition, i.e., three each of undried (29-30% of plant dry matter), partly dried (40-41% of plant dry matter), and partly dried plus water (29-30% of plant dry matter) materials. [c] Each value is a mean ± standard error.

was calculated from these measurements by difference. From these measurements and those on the intact silage, insoluble ammonium nitrogen and nonammonium nitrogen were also calculated by difference. So that it could be ascertained whether some nitrogen of the extract might be evident as protein, certain aliquots were also treated with sulfosalicylic acid (Bergen et al., 1974), and subsequent to being allowed to stand 16–18 h at 2 °C and then being filtered through quantitative filter paper, the mixtures were analyzed for total nitrogen (Association of Official Agricultural Chemists, 1950). Any difference in such content from that of the original would represent protein.

The statistical procedures (Steel and Torrie, 1960) included the analysis of variance test on the total, ammonium, and nonammonium nitrogen measurements at prefermentation as well as regression analysis on the total nitrogen measurements at prefermentation.

RESULTS

The supplementation of chopped corn plants with ammonium hydroxide to supply nitrogen at different levels resulted in an increase in the total nitrogen ($P < 0.01$) and in its fractions, i.e., in ammonium ($P < 0.01$) and in nonammonium ($P < 0.01$) nitrogen (Table I). Differences because of batch or preparation of material were not significant. The relationship of total nitrogen to added nitrogen was linear, the regression coefficient plus standard error of the data being 0.92 ± 0.06. When the data were analyzed separately for the undried, partly dried, and partly dried plus water materials, the regression coefficients plus standard errors were 0.90 ± 0.08, 0.89 ± 0.04, and 0.96 ± 0.06, respectively.

The increase in the average total nitrogen content of the chopped corn plants because of nitrogen addition was represented in large part as ammonium nitrogen and in small part as nonammonium nitrogen (Table I). However, the level of added nitrogen used in treatment was a factor in the average distribution of the increase. Whereas such distribution was 96–97% as ammonium nitrogen and 3–4% as nonammonium nitrogen in response to a 0.31 or 0.62% level of nitrogen addition, the distribution at a 0.93% level was 91 and 9%, respectively, and at a 1.24% level was 87 and 13%, respectively. Further results with prefermentation (day 0) samples of one batch (Table II) showed that the ammonium nitrogen (AN) was highly soluble in the citrate buffer extract (SAN) and that the increase which occurred in the nonammonium nitrogen fraction (NAN) because of nitrogen addition was largely accounted for as soluble nonammonium nitrogen (SNAN).

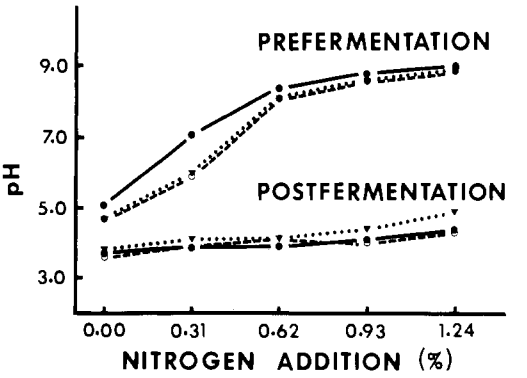

Figure 1. pH of corn silage as affected by nitrogen addition as ammonium hydroxide with undried (●), partly dried (▼), or partly dried plus water (○) material of batch 1.

When sampled at postfermentation (day 45), the ammonium nitrogen results of Table II were in good agreement with those at day 0 except for being slightly increased. Also, the ammonium nitrogen remained highly soluble. The nonammonium nitrogen values appeared to be slightly reduced at day 45, especially in the absence of a nitrogen addition or with an addition at a 0.31 or 0.62% level. Noticeable change was evident in the distribution of the soluble (SNAN) and insoluble (ISNAN) components of the nonammonium nitrogen (NAN), however. This effect, which amounted to a reduction in the insoluble component and to an accompanying increase in the soluble component, was most noticeable in the absence of a nitrogen addition. In this case, the reduction of the insoluble component (ISNAN) with the samples of undried, of partly dried, and of partly dried plus water material amounted to 34, 22, and 26%, respectively, of their day 0 NAN. In the presence of nitrogen addition at a level of 0.93 or 1.24%, the average reduction in ISNAN with the undried, partly dried, and partly dried plus water materials was 7, 10, and 12%, respectively, of the day 0 NAN of the control (untreated) samples.

The effect of ammonium hydroxide nitrogen on pH of the batch 1 samples is shown in Figure 1. At prefermentation, the pH averaged 4.8 in the absence of treatment and averaged 8.2 and 8.9 after nitrogen additions of 0.62 and 1.24%, respectively. At postfermentation, the pH values with nitrogen additions of 0, 0.62, and 1.24% averaged 3.7, 4.0, and 4.5, respectively, which showed that ammonium hydroxide treatment was effective in increasing the pH attained during ensiling.

The production of lactic acid in fermentation was noticeably increased because of ammonium hydroxide treatment, as shown with batch 1 samples at day 45 (Figure 2). Treatment at a 0.62% level of ammonium nitrogen gave a maximum response with the undried and partly dried materials which showed lactic acid increases of 45 and 52%, respectively. Either a 0.62 or 0.93% level of nitrogen addition gave a high response with the partly dried plus water material where the increase was as much 64%. Results of the acetic acid measurements, which are also shown in Figure 2, indicated that 0.31 and/or 0.62% levels of nitrogen addition were slightly stimulatory to the production of this acid, but that higher levels were either without effect or caused depression. With the content of propionic and butyric acids generally being low, i.e., less than 0.06 and 0.02% on a dry basis, respectively, those data are not cited other than to mention that two samples were exceptions. In this case, butyric acid contents of 0.38 and 0.67% on a dry basis were obtained with the undried and partly dried plus water materials, respectively, when the

Table II. Nitrogen Studies with Corn Silage of Different Preparations Which Received Added Nitrogen as Ammonium Hydroxide

preparation	added nitrogen, %[b]	days of ensiling	nitrogen of different fractions,[a] %[b]								
			total N	AN	NAN[c]	SN	SAN	SNAN[d]	ISN[e]	ISAN[f]	ISNAN[g]
nondried[h]	0.00	0	1.14	0.03	1.11	0.29	0.03	0.25	0.86	0.001	0.86
	0.31	0	1.40	0.30	1.10	0.58	0.30	0.28	0.82	0.002	0.82
	0.62	0	1.71	0.59	1.11	0.91	0.60	0.31	0.80	-0.003	0.80
	0.93	0	1.99	0.85	1.14	1.19	0.84	0.36	0.79	0.014	0.78
	1.24	0	2.32	1.08	1.24	1.48	1.07	0.41	0.84	0.012	0.83
partly dried[h]	0.00	0	1.17	0.03	1.14	0.28	0.03	0.25	0.89	-0.003	0.89
	0.31	0	1.38	0.30	1.08	0.54	0.30	0.24	0.84	0.002	0.84
	0.62	0	1.62	0.55	1.07	0.81	0.54	0.27	0.82	0.012	0.80
	0.93	0	1.99	0.79	1.19	1.12	0.78	0.34	0.87	0.012	0.86
	1.24	0	2.19	1.00	1.19	1.34	0.98	0.36	0.85	0.019	0.83
partly dried plus water[h]	0.00	0	1.09	0.04	1.05	0.27	0.05	0.22	0.82	-0.005	0.82
	0.31	0	1.45	0.31	1.14	0.60	0.33	0.27	0.85	-0.019	0.87
	0.62	0	1.79	0.59	1.20	0.90	0.59	0.31	0.89	0.002	0.89
	0.93	0	2.07	0.85	1.23	1.18	0.82	0.36	0.89	0.027	0.86
	1.24	0	2.36	1.07	1.29	1.45	1.05	0.41	0.91	0.020	0.89
nondried[h]	0.00	45	1.09	0.07	1.01	0.61	0.07	0.53	0.48	0.001	0.48
	0.31	45	1.44	0.34	1.10	0.83	0.34	0.49	0.61	0.002	0.61
	0.62	45	1.69	0.61	1.08	1.01	0.60	0.41	0.68	0.014	0.66
	0.93	45	2.10	0.90	1.20	1.34	0.88	0.46	0.76	0.028	0.73
	1.24	45	2.36	1.10	1.26	1.61	1.06	0.55	0.75	0.038	0.72
partly dried[h]	0.00	45	1.11	0.06	1.05	0.47	0.06	0.42	0.64	-0.002	0.64
	0.31	45	1.31	0.32	0.99	0.65	0.31	0.34	0.66	0.007	0.65
	0.62	45	1.49	0.57	0.92	0.91	0.55	0.35	0.58	0.011	0.57
	0.93	45	1.98	0.84	1.15	1.24	0.82	0.42	0.75	0.016	0.73
	1.24	45	2.26	1.02	1.24	1.51	0.99	0.51	0.76	0.027	0.73
partly dried plus water[h]	0.00	45	1.06	0.06	1.00	0.50	0.06	0.44	0.56	0.006	0.55
	0.31	45	1.43	0.34	1.08	0.72	0.34	0.38	0.71	0.005	0.70
	0.62	45	1.74	0.65	1.09	0.99	0.63	0.36	0.75	0.023	0.73
	0.93	45	2.02	0.87	1.15	1.26	0.83	0.43	0.76	0.034	0.72
	1.24	45	2.33	1.09	1.24	1.52	1.05	0.47	0.81	0.037	0.77

[a] Abbreviations: AN = ammonium N; NAN = nonammonium N; SN = soluble N; SAN = soluble ammonium N; SNAN = soluble nonammonium N; ISN = insoluble N; ISAN = insoluble ammonium N; ISNAN = insoluble nonammonium N. [b] In reference to the day 0 plant dry matter. [c-g] These measurements were calculated as follows: (*c*) total N minus AN, (*d*) SN minus SAN, (*e*) total N minus SN, (*f*) AN minus SAN, and (*g*) NAN minus SNAN. [h] Each preparation was from batch 1. As put up for ensiling, the samples of undried, partly dried, and partly dried plus water materials contained 30, 40, and 30%, respectively, of plant dry matter.

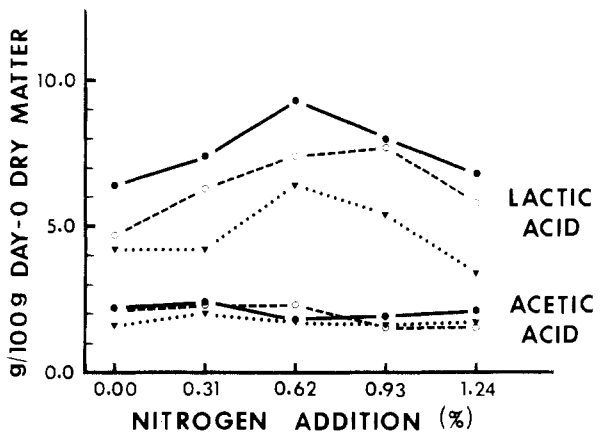

Figure 2. Effect of level of nitrogen addition as ammonium hydroxide in corn silage on lactic and acetic acid production with undried (●), partly dried (▼), or partly dried plus water (○) material of batch 1 and with the results expressed on a prefermentation basis, i.e., as percent of the original plant dry matter.

nitrogen addition was at 1.24%.

DISCUSSION

In numerous aspects, the results of the present study were in agreement with the findings of Huber et al. (1979). This concerned a linear increase in total nitrogen content of chopped corn plants or silage in response to nitrogen addition at 0.00–1.24% of the dry matter and as ammonium hydroxide. The representation of the increase as ammonium and nonammonium nitrogen was largely as the ammonium form although the proportion as the nonammonium form increased somewhat when the nitrogen addition was at a 0.93 or 1.24% level as compared to at a 0.31 or 0.62% level. Also, the results showed that ammonium hydroxide treatment reduced breakdown of insoluble nitrogen, i.e., proteolysis, during ensiling. Bergen et al. (1974) have reported that proteolysis is greatest during the first 12 h of ensiling and that ammonia treatment may interfere with this response.

The finding of Huber et al. (1979, 1980) that the ammonium nitrogen of treated samples was partly insoluble on extraction with distilled water differed from our result which showed that an extraction made with a slightly acidic citrate buffer solution gave a high solubility. In each case, the separation of soluble from insoluble matter was by centrifugation, but the procedure of Huber et al. (1979) was at 20000*g* and that of our laboratory was at 6900*g*. In this regard, an especial experiment in our laboratory to compare distilled water against citrate buffer solution for extraction and 20000*g* against 6900*g* for centrifugation, and when using material at postfermentation, did not demonstrate other than a high ammonium nitrogen extraction with all of these conditions (data not presented). Neither did filtration through quantitative filter paper when without or with sulfosalicylic acid treatment cause reduction of the extract nitrogen. These results, which were indicative of the existence of the ammonum nitrogen largely in soluble, nonprotein form, did not agree with the result of Huber et al. (1979, 1980) that ammonium nitrogen is partially insoluble in corn silage. However, factors which affect the ensiling reactions, and which are not well un-

derstood as regards the presence of added ammonium hydroxide, may be the basis of this difference.

The use of material of batch 1 which was undried, partly dried, or partly dried plus water affected the chemical composition of the silage at postfermentation. A lower conversion of insoluble nonammonium nitrogen to soluble nonammonium nitrogen was evident with partly dried material (40% dry matter) than with undried material (30% dry matter) as shown with control (untreated) samples. Also, higher pH values and lower lactic and acetic acid contents were shown with the partly dried material than with the undried material, either in the absence or in the presence of a nitrogen addition. Water addition to the partly dried material to restore the moisture content to that of the undried material was not completely effective against the effects from drying. Furthermore, an abnormal butyric acid production in samples containing 30% dry matter and 1.24% added nitrogen was not evident when the dry matter content was at 40%. Other investigators (Huber et al., 1973) have reported higher pH values and lower lactic acid contents with silage of high dry matter content.

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Nutritive Value of Fillets and Minced Flesh from Alaska Pollock and Some Underutilized Finfish Species from the Gulf of Mexico

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The nutritional properties of fillets and minced flesh from Alaska pollock and nontraditional finfish species from the Gulf of Mexico were investigated. Based on protein efficiency ratio, net protein ratio, amino acid chemical score, and protein digestibility, the data indicated that minced fish flesh, prepared by proper mechanical deboning of dressed carcasses, contained protein of equal nutritional properties to that of fillets. Significantly lower PER and NPR values were obtained for minced flesh produced by mechanical deboning of croaker filleting waste under high pressure. From a nutritional point of view, this study has shown that mechanical deboning is a suitable technology for converting the shrimp by-catch into products for human consumption.

During commercial trawling for shrimp in the Gulf of Mexico, a large amount of small bottom fish are caught with the shrimp. Even though this incidental catch, referred to as by-catch, represents a significant amount of protein, it is presently discarded back into the water. Bullis and Carpenter (1968) stated that a latent sustainable catch of 2.6 million metric tons per year of trawl fish is available from Gulf Coast waters. They estimated a total discarded by-catch of 592000 tons during the 1967 shrimping season.

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One major obstacle for the development of the by-catch as a food resource is the size and shape of the fish species normally caught in shrimp trawls. Meinke (1974) reported that landed fish average 17 cm in length and weigh around 60 g. These fish are too small to be processed by traditional techniques, and special processing procedures will be required to produce products for human consumption.

Mechanical deboning equipment currently used to recover edible flesh from poultry, meats, and fish can also be used for species which are typical for the shrimp by-catch. Mechanical deboners are not only ideal for handling these small fish, but product yield as compared to that from conventional ways of processing fresh fish is also high (Finch, 1970; Miyauchi and Steinberg, 1970; Rasekh and Waters, 1979). Finne et al. (1980) described a potential processing scheme for minced fish flesh from Gulf of Mexico finfish species.

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