Protein Concentrates

Use of Residues as Silage

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Studies were made of the ensilaging of the residue left after extraction of leaf protein from green plant materials—beet tops, carrot tops, potato veins, lima bean vines, and alfalfa. Such residues could produce good silage, readily eaten by cows. The volatile fatty acid composition and the more rapid drop in pH with alfalfa residues suggest that residues remaining after protein extraction made better silage than the starting field-chopped plant material. Pulping the plant material involved in the leaf protein extraction process may give a better distribution of bacterial nutrients and release of plant enzymes involved in the production of lactic acid. Use of waste residue by-products in the concentration of leaf protein is discussed.

Leaf protein concentrates hold great promise as a future protein source. Leaf protein can be of high quality from a nutritional point of view (Oelshlegel *et al.*, 1969). More attention should now be directed to economic and engineering considerations. The use and processing of leaf protein concentrates on a large scale will take place more rapidly if equipment and products can be integrated into present agricultural practices.

One study (Akeson and Stahmann, 1964, 1966), indicated that protein concentrates from alfalfa will yield more protein per acre than seed crops. The use of animals as a protein source is much less efficient than use of plants directly. Waste green crops such as beet tops, pea vines, etc., can be used in the leaf protein process (Oelshlegel *et al.*, 1969).

Utilization of the by-products of leaf protein preparations would lower the over-all costs. The plant residues left after the protein is extracted can been silaged and used as a ruminant feed (Pirie, 1956). However, we are not aware of any published report of ensilaging such residues into a material acceptable to ruminants. In fact, earlier unpublished attempts in this laboratory to ensilage alfalfa residue in 55-gallon drums were not successful. The residue spoiled, probably because of air leakage. In the present study, we used plastic bags to maintain anaerobic conditions and demonstrated that residues from the leaf protein extraction procedure can be ensilaged into a material readily eaten by dairy cows.

MATERIALS AND METHODS

The plant material was obtained by procedures used previously (Oelshlegel *et al.*, 1969). Unchopped alfalfa was obtained from the field by cutting the plants at the base of the stem. Residue refers to alfalfa harvested with a field chopper and pulped and pressed for leaf protein (Hartman *et al.*, 1967). Some field-chopped material and residue were sun-dried for about 3 hours to get the low moisture samples used.

Plant material to be ensilaged was placed in Cryovac plastic bags obtained from W. R. Grace and Co., Cedar Rapids, Iowa. The bags were then evacuated with a water aspirator to compact the material and tied tightly with a string. The bags puffed up considerably for the first 3 days but did not break. They were left at room temperature until used. For the animal feeding studies large bags containing 16 to 20 kg. (35 to 45 pounds) of sample were used. Smaller bags containing about 1 kg. of material were used for other studies.

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For pH determinations, a sample of silage was placed between two aluminum plates 1/4 inch thick and 4 inches square, covered with Parafilm, and squeezed in a vice. The juice squeezed out was collected in a beaker and the pH measured.

Volatile fatty acids were determined by placing 30 to 50 grams of plant material in a Waring Blendor and adding water. (Usually the volume in milliliters was four times the weight in grams of the material used.) The blender was run at high speed for 2 minutes. The resulting pulp was squeezed through a double layer of cheesecloth. The expressed juice was then centrifuged on a Sorval angle centrifuge for 5 minutes at 2250 r.p.m. (about 800 G). Five milliliters of the resulting supernatant were pipetted into another centrifuge tube, to which was added 1 ml. of 25% metaphosphoric acid. The tube was agitated, allowed to stand for 30 minutes, and then centrifuged for 20 minutes at 3020 r.p.m. (1500 G). The final supernatant was used for volatile fatty acid analysis.

Volatile fatty acids were determined by a gas chromatographic method very similar to that routinely used by the Dairy Science Department (Baumgardt, 1964). A Hewlett-Packard Model 402 gas chromatograph with a hydrogen flame detector was used.

To make the column packing, 10 grams of firebrick (60- to 80-mesh) was placed in a round-bottomed flask and 2 grams of neopentyl glycol succinate (NPGS) dissolved in chloroform was added. The flask was shaken 30 minutes and the chloroform evaporated with a rotating evaporator. To the dry residue was added 0.118 ml. (0.2 gram) of 85% orthophosphoric acid dissolved in 70% ethanol. This was shaken for 30 minutes, evaporated to dryness, and heated at 110° C. for 1 hour. This packing was then placed in a 6-foot U-shaped column (1/8-inch i.d.), which was conditioned overnight at 220° C.

Five microliters of sample were usually injected. Carrier gas flow (N_2) was 25 ml. per minute, H_2 flow was 20 ml. per minute, and air flow was 160 ml. per minute. Oven temperature was 165° C., and flame detector and flash heater temperatures were both set at 195° C. Usually range 10 and attenuation 16, 32, or 64 were used. The order of elution was acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids with good resolution. The last acid, valeric, was eluted in 5.5 minutes. After every three runs, water was injected twice to prevent "ghost" peaks from appearing (Metcalfe, 1963). When numerical values could be obtained, area of peak (height × width at half height in millimeters) was measured and compared with a standard curve. In many cases the peak areas were too small to be accurately measured, so (+) was used if a

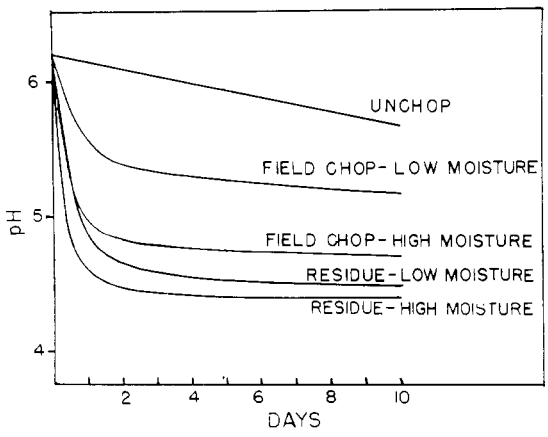

Figure 1. pH of alfalfa silage

peak was definitely present and (−) if not. The amount of fatty acid in the original material was calculated taking into account both the water originally present and that added.

Moistures were determined by azeotropic distillation with toluene. About 25 grams were used for analysis.

RESULTS AND DISCUSSION

Proximate analysis and yields for the vegetable waste residues have been given (Oelshlegel *et al.*, 1969). With good equipment, at least half of the protein can be readily extracted from plant materials, leaving half in the residue. If such residues prove too low in nitrogen for feeding cows, supplementation with urea is feasible. Some workers have used up to 58% nitrogen supplementation in the form of urea or ammonium sulfate for zebu bulls (Preston *et al.*, 1967). One study used urea as the sole source of nitrogen; here, however, the animals grew to about $^1/_2$ to $^2/_3$ normal size (Mattson, 1965). Virtanen (1969) has reported on nitrogen metabolism and milk production from cows fed rations in which urea was the sole source of nitrogen. In such an extreme case the materials used are not competitive with a foodstuff for the human population.

The moisture content of some of the residues was over 80%. Material with so high a moisture content, if used to make ordinary silage, would probably spoil because of the activity of clostridial microorganisms (McDonald *et al.*, 1968). In the normal silage process clostridial organisms are inhibited by low moisture and low pH—i.e., acidic fermentation (Whittenbury *et al.*, 1967). In Figure 1 are shown results of a pH study with alfalfa silage. Moisture of the various samples was: unchopped 81.7%; field-chopped, high moisture, 81.7%; residue, high moisture, 80.5%; field-chopped, low moisture, 69.7%; residue, low moisture, 74.2%. Both residues showed a more rapid pH drop and leveled off at a lower pH than either field-chopped sample and the pH's for the high moisture samples were lower than their low moisture counterparts. There is a definite relationship between pH and lactic acid concentration (Barnett, 1954). Lactic acid is principally responsible for the drop in pH of silage. Thus with both high and low moisture residues, there is a more rapid and greater production of lactic acid than for the field-chopped samples.

The presence of clostridial organisms is harmful in silage. Clostridial species are harmful because they decarboxylate amino acids to amines which endanger the health of animals eating such silage (Whittenbury *et al.*, 1967). One indicator of clostridial activity is the production of butyric, isobutyric, isovaleric, and caproic acids (McDonald *et al.*, 1968). The experiments summarized in Table I indicate that there was little or no clostridial growth in our residues. The field-chopped material, on the other hand, seems to have experienced clostridial attack. These results are consistent with our pH study. Thus the drops in the pH's of the high or low moisture residues stopped clostridial growth, whereas in the field-chopped material the pH's did not fall rapidly or low enough.

We also attempted to study heat development in silage by placing Cryovac bags containing 1 kg. of material in 4-liter Dewar flasks. Temperatures were recorded continuously for 5 days with thermocouples placed in the bags. While all samples showed some heat buildup during the first day, no differences were observed between silage from the alfalfa residues and from chopped alfalfa.

Our results suggest that the pulping of plant material has a beneficial effect in silage making. de Man (1952) noted that grass crushed with a small roller mill gave a silage of lower pH than uncrushed grass. He attributed this to a better distribution of stem carbohydrates. When grass is crushed, there is probably a more rapid growth of silage bacteria and hence

Table I. Volatile Fatty Acids Found in Alfalfa Silages[a]

Sample	Time, Weeks	Acetic Acid, μl./G.		Other Acids, μl./G. Fresh Wt.				
		Fresh wt.	Dry wt.	Propionic	i-Butyric	Butyric	i-Valeric	Valeric
Unchopped alfalfa	0	+	+	−	−	−	−	−
	3	10.9	59.5	+	?	+	+	−
	5	11.2	62.0	+	?	+	+	+
Field chopped alfalfa, high moisture	0	+	+	−	−	−	−	−
	3	8.1	44.5	+	−	1.13	+	+
	5	9.4	51.0	+	+	+	+	+
Residue alfalfa, high moisture	0	+	+	−	−	−	−	−
	3	9.4	48.5	+	−	?	−	−
	5	8.5	43.6	+	−	?	−	−
Field chopped alfalfa, low moisture	0	1.0	3.3	−	−	−	−	−
	3	7.2	23.8	+	+	+	+	−
	5	10.9	36.0	+	?	+	+	−
Residue alfalfa, low moisture	0	1.6	6.2	−	−	−	−	−
	3	10.7	41.7	+	−	−	−	−
	5	12.3	47.7	+	−	−	−	−

[a] + peak present, − not present, ? small doubtful peak.

Table II. Silage Feeding Trials (1967)[a]

Sample	Starting Moisture, %	Storage Period, Days	Consumed, %
Lakeweeds	76	76	1
Beet tops	82	67	100
Carrot tops	82	14	80
Potato vines	85	33	100
Lima bean vines	61	28	100
Alfalfa	84	125	100
Alfalfa	73	127	100
Alfalfa[b]	74	141	100
Alfalfa[c]	84	300	95

[a] Silage fed to heifer 1594H. One half of bag fed in A.M., other half in P.M. Material not eaten was weighed and amount consumed calculated.
[b] Fed to four Holsteins.
[c] Fed to milk cow over 7-day period, with no decrease in normal milk production. Total amount of alfalfa used 139 kg. (306 pounds).

greater anaerobic fermentation. Perhaps there is also a better distribution of plant cell enzymes and substrates. Plants are known to contain all the enzymes needed to metabolize hexoses to lactic acid (Bonner and Varner, 1965). In breaking up the cells we release enzymes (and substrates) which participate in the breaking down of hexoses to lactic acid under the anaerobic conditions present. Thus the plant enzymes may participate in the fermentation process along with the normal silage bacteria, resulting in a more rapid pH decrease and a lower final pH than with uncrushed plant material. The removal of some soluble buffers may facilitate the pH drop in the residues.

We can conclude that residues remaining after processing plants for leaf protein can be used for silage making. Our process gave a high moisture residue; it may be desirable to design equipment to press out more water and give a lower moisture residue, since with a low moisture residue a higher dry matter intake is possible and there is less seepage loss in the silage process (Gordon *et al.*, 1965).

We have chosen to make silage out of our forage residues. The residues could be fed fresh in areas where forage crops are grown most of the year, or dehydrated to make products similar to dehydrated alfalfa (Hartman *et al.*, 1967). Silage made by such a process would be relatively independent of weather conditions, compared with hay making.

The data of Table II show that from several waste plant residues and alfalfa residue good silage was obtained which was readily eaten by dairy cows. Only the silage from the residue of lakeweeds was rejected by the cows. In one experiment, silage from our alfalfa residue was fed to a milk cow for one week with no significant reduction in milk production. In larger studies of macerated, dewatered *vs.* wilted alfalfa-grass silage for dairy cows, Hibbs *et al.* (1968) report no difference in milk production from the dewatered silage and wilted silage; the over-all efficiency of utilization of the digested dry matter and the per cent of feed nitrogen converted to milk were greater in the cows fed dewatered silage. Derbyshire *et al.* (1967) reported similar animal performance and digestibility from alfalfa forage ensiled as direct-cut, wilted, and mechanically dewatered material.

In all these experiments, about half of the forage protein was removed before the residue was ensiled. However, the juice containing about half the alfalfa protein was discharged onto the ground. The yields and protein content of these juices seem similar to those obtained in our study (Oelshlegel *et al.*, 1969). The implications for leaf protein work are obvious. With only minor modifications to collect the juice rather than allowing it to drop to the ground, this equipment could serve as a source of large amounts of leaf protein along with dewatered alfalfa. The protein in the juice could be recovered and the residue fed to animals to produce milk and meat. It would thus be possible to integrate protein production from green plants into our present agriculture with a benefit to the dairy and beef farmers, the meat-consuming public of developed countries, and the protein-hungry masses of the underdeveloped countries.

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