ASSAYS FOR DETERIORATION AND ESTIMATES OF FEEDING VALUE OF CORN SILAGE

by

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LITERATURE REVIEW

SILAGE FERMENTATION

Silage fermentation is important to the preservation of forages with respect to feeding value and animal performance. Chemical and bacteriological changes which occur in the silo during the fermentation process profoundly affect nutrient yield and quality (51). Abnormal fermentations are detrimental to conservation of the forage crop and may reduce feeding value (81).

Commonly accepted criteria for evaluating the desirability of silage fermentation have included pH, color, taste, aroma, and texture (58). These indicators of fermentation type may or may not relate to actual animal performance (58).

Normal Fermentation

A general discussion of characteristics of a normal fermentaion will aid in understanding the lowering of forage quality by abnormal fermentations. Langston et al. (41) found that certain bacteriological and chemical variations determined the course of fermentation during the early stages of ensiling. They concluded that the characteristics of

a normal fermentation would include: rapid production and high levels of lactate, low levels of butyrate, acetate, and ammonia nitrogen, and low numbers of sporeforming anaerobes (41).

The first objective of the ensiling process is to achieve and maintain anaerobic conditions and secondly, to discourage deleterious clostridial growth (51). If these objectives are met, the ensiled material should follow five phases of fermentation, as described by McCullough (49). In the initial phase of silage fermentation, plant cell respiration removes oxygen from the crop material with the production of carbon dioxide and heat, resulting in a temperature rise of the entire mass of material (49). Therefore, the first phase is important to establishing anaerobic conditions. In phase two, glucose, fructose, sucrose, and fructosans provide the herbage water-soluble carbohydrates (WSC) which serve as the major substrate for initial bacterial growth (51). As the fermentation moves into phase three, a rapid explosion in bacterial growth should occur and under favorable conditions, lactic acid bacteria will dominate, resulting in the production of lactate, acetate, mannitol, ethanol, and carbon dioxide (83). In phase four, lactate production continues with a resulting decline in pH to around 4.0 (49). If the above events have taken place

without much variation, the silage should maintain a fairly constant composition until it is removed from the silo for feeding (49).

Representative values obtained for dry matter, pH, volatile nitrogen and organic acids for various species of silage and several ensiling methods are given in Table 1. Considerable variation in these components has been observed even in silages which have undergone what would be considered normal fermentations.

Abnormal Fermentations

The clostridial type of fermentation occurs when conditions in the silo are favorable for the rapid growth of anaerobic Clostridium. Clostridial activity has two deleterious effects on silage quality: 1) production of butyrate from lactate and decarboxylation and deamination of amino acids contribute to greater energy and dry matter losses in the form of evolved carbon dioxide and ammonia (17) 2) The production of certain amines (cadaverine, putrescine, histamine, gamma-amino butyrate, beta-alanine, tyramine, and tryptamine) may affect animal health and voluntary intake (58, 83).

Inhibition of clostridial activity is accomplished by reduction of the moisture content of the crop and promotion

of an acid type fermentation (83). Clostridia are very sensitive to osmotic pressure, and require wet conditions (less than 30% DM) for active growth (51). Increased hydrogen ion concentration and organic acids from an acid fermentation will also inhibit clostridial growth (51).

It is well established that silage fermentation is carried out by a small proportion of microorganisms initially present on the fresh material (45, 51, 83). In order for proper fermentation to occur, the lactic acid bacteria must demonstrate a superior competitive ability for available substrates in order to precipitate a rapid pH decline. Kempton and Clemente (45) found an initial proliferation of lactic acid bacteria in both well preserved and spoiled silages. However, the lactate was replaced by butyrate in spoiled silages beginning about day four of the fermentation and continuing over the next three to four weeks. Several factors including dry matter, buffering capacity, and substrate availability could contribute to the conversion of lactate to butyrate as the fermentation progresses (50, 51, 83).

Table 1. Dry matter, pH, volatile nitrogen, and organic acids of various silages and silo types.

Silage and silo type	DM%	pH	NH_3/N (% of N)	Acetate	Propionate	Butyrate	Lactate	Reference no.
				% of DM				
Alfalfa-brome[a] concrete stave	27.2	5.3	27.6	4.5	0.63	2.99	3.95	(68)
Alfalfa gas tight steel	26.6	4.9	16.1	5.6	0.40	0.60	2.20	(28)
Hay crop sealed bunker	18.5	4.8	21.1	6.1	1.50	3.31	2.40	(29)
Ryegrass straw laboratory silo	27.8	3.9	--	5.6	--	0.80	2.70	(73)
Italian ryegrass[a] metal silo	34.0	4.2	16.6	5.5	0.15	nil	2.10	(52)
Italian ryegrass metal silo	15.9	3.7	22.0	12.0	0.13	nil	3.60	(52)
Orchardgrass laboratory silo	17.9	4.8	--	3.6	0.12	0.10	3.90	(22)
Alfalfa laboratory silo	41.9	4.6	--	1.3	1.66	0.67	3.66	(64)
Orchardgrass laboratory silo	--	3.8	1.2[b]	1.5	--	--	8.75	(41)
Grass and legumes	21.5	4.0	--	2.8	nil	nil	17.90	(50)
Alfalfa laboratory silo	--	4.5	2.4[b]	4.0	--	--	6.58	(41)

[a]Crop wilted prior to ensiling.

[b]Reported as % of dry matter.

Low Dry Matter Silage

Disadvantages of ensiling low dry matter forage (i.e. less than 30% DM) include : 1) greater nutrient losses from undesirable fermentations (52) 2) higher nutrient losses from silo seepage (25) 3) lowered animal intake (12).

Dry matter content of the ensiled material will have a profound effect on the type and extent of fermentation (52). Clostridia are sensitive to moisture content and their growth is normally inhibited in silages above 30% DM (51). Furthermore these bacteria are less sensitive to a given concentration of organic acids in a low dry matter silage (14). Consequently, a rapid and substantial drop in pH is generally of lesser importance for preserving high DM than for low DM silage.

Gordon et al. (27, 28) ensiled alfalfa (20-53%DM) in gas tight structures. Dry matter losses were 4 and 24% for the high and low DM silages respectively with very little spoilage, which indicates mainly seepage and gaseous losses (28). McDonald et al. (52) ensiled Italian ryegrass fresh (16% DM) and wilted to 30, 34, and 47% DM. Total DM losses were 16% for the fresh grass compared with 2 to 4% for the wilted silage. All silages were well preserved with the greatest losses being effluent and gas (52). Although many factors may be involved in silo losses, Gordon (25) suggests

that dry matter losses as high as 20% can be expected when ensiling low dry matter crops.

Silage is consumed to a lesser degree by ruminants than is the same crop fresh or preserved at higher DM (18, 26, 28, 58). There is a positive linear correlation between DM of silage and voluntary intake over the range of 30 to 70% DM (26, 31). Several factors including: DM, pH, increased osmolality of rumen liquor, products of protein breakdown, changes in rumen motility, and decreased rate of ruminal fermentation have been suggested as causative agents for decreased intake of low DM silage (81). Vetter and Von Glan (81) suggest that moisture *per se* is not the causative agent for lowered intake, but the correlation does exist.

Gordon et al. (28) compared alfalfa silage from direct-cut (24% DM), heavily wilted (53% DM) silage, and hay (89% DM) in feeding trials for three consecutive years. Dry matter consumption by cows was significantly lower for the direct cut versus wilted silage or hay in years two and three (28). Later, Gordon et al. (26) ensiled alfalfa at three different average dry matters in two successive years with silages ranging from 39 to 65% DM. Dry matter consumed ranged from 13.9 to 41.7 lb/day for all six silages. The correlation (r) of DM consumed and DM of the silage of .53 was significant ($p<.01$) (26).

High Dry Matter Silage

Since the introduction of gas tight silos there has been a substantial increase in the practice of wilting grasses and legumes prior to ensiling. The practice of wilting crops to DM above 30% prior to ensiling will reduce storage losses, discourage clostridial growth, and increase animal intake (28). Improvement in silage quality and preservation by wilting hay crop silages to 65-75% DM has been reported by many investigators. Dry matter is an important consideration related to quality because laboratory analyses indicate that high DM silages undergo limited fermentation relative to direct-cut ensiled grasses and legumes (26, 28, 68).

When progressively higher DM silages are well preserved (ie. oxygen has been excluded in either gas-tight steel structures (28) or concrete stave silos (26, 68) almost no difference in pH from direct-cut silages was observed. This was true even when total acid production was higher for low DM silage (28). Gordon et al. (26) reported higher production of acetate, butyrate, and propionate for 20% DM compared to 53% DM alfalfa silage. Lactate was twice as high for the 53% DM silage. Dry matter consumption tended to be increased for the higher DM, but neither milk nor fat corrected milk yield were affected by DM of the silage (26).

These results were comparable to the findings of Roffler et al.(68). Contrary to the above results, Owen and Sengel (64) found higher pH and lower lactate for 53% DM versus 35% DM alfalfa silage in laboratory silos. However, propionate and butyrate were higher for the 35% DM silage (64).

In each of the above studies ammonia nitrogen (NH_3/N) values were considerably higher in the low DM silages (26, 68). Roffler et al. (68) reported ammonia nitrogen as a percent of total nitrogen of 27.6, 24.2, and 16.4% for low DM silage versus 5.5, 8.7, and 5.6% for high DM silage in three trials, respectively. Decreased NH_3/N for high DM silages would indicate limited fermentation or loss of NH_3/N to the environment.

High DM silages are more susceptible to heat damage (24, 33, 68). Roffler et al. (68) have shown that digestibility of nitrogen is reduced for high DM alfalfa-brome silage. Hill and Noller (33) also showed lower apparent digestibility of nitrogen in high DM silages. Oxygen exclusion is critical when ensiling high DM forage to prevent heat damage from occurring (67).

Oxygen

A normal lactic acid fermentation depends on anaerobic processes to rapidly reduce pH and terminate undesirable plant respiration. Continued plant respiration results in DM losses and damaging temperatures (23, 24, 51). Related to silage quality there are three critical periods of oxygen exposure: 1) aerobic phase in the silo 2) air infiltration during fermentation 3) air exposure when feeding.

Oxygen inclusion during the ensiling process is unavoidable. According to Ruxton et al. (69) calculations show that the volume of air trapped in properly sealed silos is not sufficient to cause departure from the course of normal fermentation. Rapid filling and sealing of the silo is important to a satisfactory fermentation. Greenhill (30) has shown that anaerobic conditions are a prerequisite for plant cell breakdown and release of plant juices for bacterial growth.

Miller et al. (54) ensiled a mixture of ryegrass and crimson clover to compare rapid-filling (one day) and slow-filling (4 days). Chemical composition of the two silages was similar but a slightly higher pH, ammonia nitrogen, and crude fiber indicated that slow-filling resulted in a lower quality silage. Silage consumption by dairy cows was fairly stable for the rapid-fill silo, but intake decreased linear-

ly over a six week period for the slow-fill silo. Dry matter digestibility was 61.5 and 57.7% for fast and slow-filled, respectively (54).

Greenhill (30) ensiled ryegrass and alfalfa in laboratory silos which were sealed immediately or sealed after 21 hours delay. Collapse of the silage was measured by a pen recorder attached to a lead weight on a sliding piston. Collapse of the immediately sealed silo began after 3 hours for ryegrass and after 2 hours for alfalfa, and was virtually complete after 10 hours. In the unsealed silos there was a gradual settling during the first 21 hours, and the rate of collapse increased substantially two or three hours after sealing. Increased electrical conductivity which indicates protoplasmic injury suggested plant cell breakdown accompanied collapse (30).

It has been demonstrated that DM losses and detrimental effects of oxygen are small if sealing is adequate (29, 41, 81). The major effect of oxygen in terms of nutrient losses and silage quality arise from air infiltration (ie. improper sealing). Brown and Kerr (9) compared losses of heavily wilted forage (approximately 50% DM) in lined trench silos with or without a polyethylene film seal. In unsealed silos, 70% of the material was inedible compared to DM losses of 8.2 and 5.2% for sealed silos (9). In a similar study

using non-wilted hay crop silage, Gordon et al. (29) reported DM losses of 37.1 and 46.9% for unsealed compared to 19.1 and 21.3% for sealed trench silos.

It is apparent that air infiltration during the fermentation period contributes to loss of nutrients and feeding value. These losses are the result of wasteful fermentations and respiration by aerobic molds, yeasts, and bacteria (81). Due to high temperatures attributable to extended plant cell respiration, DM and protein losses may result (23).

There have been no detailed investigations of the detrimental effects of oxygen at the silage feeding stage. Its importance would depend on feeding management and type of silo. O'Leary et al. (63) examined microbial stability of corn and triticale. The average log yeast and mold counts for corn were 6.99, 6.69, and 5.86 for 30%, 40% DM, and oxygen limiting silages, respectively. Silage stability was 36 hours for corn compared to 18 hours for triticale (63).

According to Ruxton et al. (69) in their review of the effects of oxygen on ensilage, silages particularly at risk at feeding time are those made from WSC rich herbages such as corn. High DM silages are also at a greater risk to aerobic deterioration because of more rapid air penetration.

Various additives have been tested as silage preservatives (40, 61, 62). O'Leary and Hemken (61) inoculated corn and alfalfa silages (35%DM) with frozen bacterial cultures and .6% propionate in plastic laboratory silos. Yeast and mold counts decreased rapidly in most silages and were less than 100/g by day 4. On exposure to air, the propionate treated silage was least stable and yeast counts had risen to >106/g by day 3. The control silage was stable for 5 days and some inoculated silages for 10 days (61). In a similar study employing stave silos , corn silage yeast and mold counts were higher for silage treated with Lactobacillus plantarium (1.3 X 106/ g) than for control silage (2.1 X 105/g) (61). Lane and O'Leary (40) treated corn silage with anhydrous ammonia (7kg/2000 kg wet silage) stored in laboratory silos. The ammonia treated silage was stable six days compared to two days for the control silage (40).

These preliminary results suggest that silage additives such as bacterial cultures and anhydrous ammonia may help extend bunk-life of silage.

Temperature

Ambient temperature appears to have limited effects on silage bacterial populations (20). It is difficult to actually separate the effects of temperature from those of oxy-

gen since a rise in temperature is the result of aerobic respiration (84). Fermentation of one mole of glucose by anaerobes results in the formation of one to two moles of ATP. On the other hand, respiration by aerobes of the same substrate results in the formation of 38 moles of ATP (53). This limited ATP production by anaerobic systems relative to aerobic systems results in less heat production and subsequent temperature increase (81).

At temperatures above 40° C, oxygen is responsible for the fixation of protein into an undigestible compound resulting in heat damaged, caramelized, or oxidized silage (84). Heat damage has been identified as the condensation of carboxy groups with amino groups of proteins, amino acids, and other compounds to form a dark colored polymer (24). This development has been termed the Maillard reaction, the nonenzymic browning reaction, and the browning reaction (24).

Apparent digestibility of dry matter and protein is lower in heat damaged forages (33, 66, 84). Wieringa et al. (84) ensiled grass in glass jars aerated for 0, 1, or 2 days at temperatures of 55°, 60°, and 65° C. There was no influence of temperature on digestibility of the crude protein, however, aeration significantly depressed digestibility at all three temperatures (84).

Goering et al. (24) studied the relative susceptibility of different forages to heat damage as affected by moisture, temperature, and pH. The extent of heat damage was estimated by determining the nitrogen content of the acid detergent fiber fraction. Acid detergent fiber nitrogen (ADF-N) increased steadily from 0 to 80 hr at 80° C. In buffered silages, ADF-N levels were higher at higher pH and heat damage increased linearly with temperature above 60° C. Very little browning was observed for temperatures below 60° C. It was also noted that moisture content above 10% was necessary for browning to occur (24).

FORAGE QUALITY EVALUATION

Defining Forage Quality

Forage quality can be defined as the usefulness of a specific forage to an animal. The primary goal of forage evaluation is the rapid, reliable, and accurate prediction of forage quality based on laboratory analyses (8). Forage quality is simply the potential of livestock to produce meat, milk, and other products from the utilization of nutrients provided by forages (8). Consequently, it is necessary to determine the characteristics of a forage which relate to animal performance and develop a numerical expression which will describe the feeding value of a particular forage.

Possibly the first attempt to define forage quality in a quantitative sense was by the apparent digestibility of total nutrients including energy (7). Although digestion data has been used extensively for a number of years for the evaluation of forages, digestibility does not consistently express the effective feeding value of a forage (15). In an attempt to further define forage quality, Crampton et al. (15) proposed the nutritive value index (NVI). The NVI combines forage intake relative to a standard forage and digestibility of the energy in the forage. When changes in body weight of sheep were regressed on NVI, correlations of .88 to .94 resulted. The correlations of the predictions by NVI were stronger than either relative intake or digestibilty of calories alone (15).

More recently Barnes and Marten (8) defined forage quality relative to the type and amount of digested nutrients available per unit time. According to Barnes and Marten, "forage quality is a function of the rate and level of intake, the rate and extent of digestion, and the efficiency of utilization of specific nutrients." The question remains how to apply numeric values to the above definition.

Indicators of Forage Quality

Barnes (7) provides a comprehensive review of methods for estimating feeding value of forages. Chemical, physical, in vivo, and in vitro rumen fermentation techniques and a comparison of methods are found in this discussion (7).

In the mid-1800's the Weende system was proposed for the proximate analysis of crude fiber, crude protein, ether extract, ash and nitogen free extract (80). This system is based on the concept that crude fiber represents the undigestible fraction and nitrogen free extract the digestible fraction of the plant (80). Van Soest (80) divided forage dry matter into fractions on the basis of nutritional availability. The first fraction includes cellular contents, which he termed as essentially available but digestion is incomplete. The second fraction includes the plant cell wall and its availability depends on structural features that link cellulose, hemicellulose, and lignin together.

Currently the Weende proximate analysis and Van Soest systems are the most commonly employed laboratory tests for describing forage quality (13). The feeding standards most often employed as forage quality indicators include total digestible nutrients (TDN), dry matter digestibility (DMD), digestible organic matter (DOM), voluntary intake of dry matter (DMI), nutritive value index (NVI), and average daily

gain (7). These methods have been extensively tested and utilized for the prediction of feeding value for grasses and legumes. Limited research is available that tests the reliability of these methods for predicting corn silage quality (47, 48, 70).

IN VITRO DIGESTIBILITY

Numerous *in vitro* rumen fermentation procedures have been proposed for the prediction of *in vivo* digestibility (70). The two-stage *in vitro* dry matter digestibility (IVDMD) introduced by Tilley and Terry (77) in 1963 has been accepted among plant-breeders and ruminant nutritionists as a practical method for predicting digestibility (75). This procedure is not limited to the prediction of DMD or DOM. Modified *in vitro* fermentation procedures have been developed to predict digestible energy (DE) (10) and NVI (19).

Various procedural modifications of the original Tilley and Terry procedure have been proposed to simplify the method (67, 76). These modified procedures approximate the Tilley and Terry method with various degrees of reliability. The lack of a standardized *in vitro* rumen fermentation method has been a major criticism of the technique (5). The high cost and dependence on a fresh supply of rumen fluid (75) and the lack of agreement between laboratories (5) have

also been cited as drawbacks for the method. The relative advantages of the *in vitro* method over *in vivo* procedures includes the rapid determination of large numbers of samples (55), the small sample size required (77), the accuracy for a wide range of feed samples (75), and the potential for determining digestibility of abnormal or even toxic feeds.

For grasses and legumes there is a strong correlation between IVDMD values and DMD (75). Tilley and Terry (77) using 146 samples of grass, clover, and alfalfa of known *in vivo* digestibility, found a correlation with IVDMD of .99 S.E.$\pm$ 2.31). The correlation was somewhat lower (r=.85) for 18 legume and clover samples (77). These results are comparable to Terry et al. (75) who found a correlation .971 between DMD and IVDMD for 73 grasses and legumes.

Schmid et al. (70) compared six biological methods for predicting *in vivo* digestibility of 26 sorghum and 25 corn silages. A modified Tilley and Terry two-stage fermentation procedure gave the highest correlations (.83 for corn silages and .91 for sorghum silages) (70).

The validity of comparing *in vitro* digestibility data from different laboratories is doubtful. Barnes (5) in a collaborative study of *in vitro* procedures among 17 laboratories, found the variance component due to labortatories was considerably greater than for either runs or determinations within runs.

CORN SILAGE

Well-eared corn silage has a very high feeding value for fattening cattle (27) and dairy cattle (46). In areas where corn is grown, corn silage has become the predominant forage for dairy cattle. There is very little information in the literature concerning chemical and bacteriological changes during the ensiling of corn. There is evidence that laboratory methods routinely employed for predicting quality of perennial grasses and legumes may be inadquate for predicting corn silage quality (48).

Maturity

Variations in digestibility of corn silage DM are small compared to other forages (34). Maturity of the whole corn plant seems to have little or no effect on the feeding value of corn silage (27, 34, 35, 36, 38). Johnson and McClure (38) harvested corn for silage at eight stages of maturity to feed to sheep. The highest DM and OM digestibilities were at the milk, early dough, and dough dent stages of maturity. Maximum digestibility corresponds roughly to maximum DM yield (38). Johnson et al. (37) reported maximum acre yield of corn silage DM occured between the dent and glaze stages.

A steady decline in *in vitro* digestibility of the whole corn plant with maturity was reported by Deinum and Dieven (16). Weaver et al. (82) found increases in grain content offset decreases in digestibility of other plant parts until the grain was physiologically mature and total digestibility began to decrease.

Gordon et al. (27) reported no differences in DM consumed, milk production, or body weight when normal and late harvested corn silages were fed to dairy cows. Huber et al. (35) found no differences in TDN content of corn silage DM or digestibility of DM components for soft dough, and hard dough corn silages. However, with increasing maturity significant increases in DM intake and milk yield were noted (35). The above findings are also consistent with the results of Owens et al. (65).

Feeding value remains relatively high for mature corn silage because of the increased proportion of the DM that is grain. Weaver et al. (82) reported 50% of the total DM as grain for mature corn silage. Huber et al. (35) found ears comprised 34, 47, and 51% of total DM for soft, medium, and hard dough maturities. In two successive years Johnson et al. (37) reported that ears comprised over 60% of total DM at maturity. Increased grain content tends to compensate for declines in digestibility of other plant parts (82).

Deinum and Dieven (16) and Weaver et al. (82) reported lower IVDMD for stalks, leaves, and husks with later harvest. Digestibilities of selected corn silages are given in Table 2.

Dry Matter

There is interest in the effects of DM on corn silage quality because extending the harvest season to later dates could help alleviate problems of timely harvest and silo capacity (27). Dry matter content would be expected to influence silage quality by altering fermentation (44) and voluntary intake (65). Dry matter content of corn silage increases with maturity (27, 35, 38).

Owens et al. (65) compared medium DM (ca. 37% DM) and high DM (ca. 62% DM) corn silages fed to lactating dairy cows. Voluntary DM consumption, milk production, and body weight gains were higher for cows fed the high DM silage. The digestibility of the DM, energy, and protein for the medium and high DM silages were 66.9, 66.7, and 54.7%; and 64.7, 65.4, and 51.1%, respectively (65). Gordon et al. (27) fed lactating cows normal (26 and 30%DM) and late harvested (58 and 63%DM) corn silage in mixed rations. No difference in feeding values were observed related to harvest date or DM content.

Table 2. Corn silage invivo and in vitro digestibility.[a]

Reference	TDN	in vivo		in vitro	
		DMD	DOM	DMD	DOM
	----------	% of DM		----------	
(39) heifers		64.8			
(39) heifers		66.1			
(43)				68.3	68.4
(35)	68.0				
(57) cows		59.3			
(65) cows		66.9			
(46) lambs			67.1		
(70)				72.0	
(82)				85.6	
(47) sheep		64.0			

[a]Abreviations, total digestible nutrients (TDN), dry matter digestibility (DMD), digestible organic matter, (DOM).

The effect of DM on type and extent of fermentation has been demonstrated by observing changes in pH, volatile acids, lactate, and volatile nitrogen. Klosterman et al. (46) reported pH of 3.7 and lactate of 6.26% for normal corn silage. This compares with pH 3.8 and lactate of 7.8% found by Gordon et. al. (27) in normal corn silage (Table 3). Lopez et al. (44) prepared low (25.6%), medium (30.4%), and high (52.0%) DM corn silage in plastic bag silos. Total VFA's and lactate were 2.61, 7.72; 1.99, 4.21; and 0.80, 2.05% of DM for low, medium, and high DM silages, respectively.

Ammonia or volatile nitrogen levels are often cited as indicators of catabolism of amino acids in silage. Gordon et al. (27) found ammonia levels of 8.46% of total nitrogen for normal compared to 6.52% for high DM silages. Lactate and total VFA production were also higher indicating higher ammonia nitrogen results from a more extensive fermentation (27).

The literature appears to indicate that DM content does affect the type and extent of fermentation. These differences do not seem to be reflected in reduced feeding value or digestibility (35, 38, 82).

Although overall silage quality may not be affected by dry matter and maturity, changes in the digestibility of

Table 3. Chemical composition of corn silage.[a]

DM%	CP	CF	NFE	EE	ADF	NDF	pH	Acetate	Lactate	Reference
	% of DM									
32.1	9.0	22.5	59.6	3.7	28.8	58.1	3.7	---	---	(48)
35.7	8.5	19.7	61.5	3.6	----	----	4.1	1.0	7.8	(39)
39.0	9.2	15.2	65.7	3.9	----	----	4.0	0.9	6.4	(39)[b]
37.6	7.9	22.8	62.4	2.1	----	----	3.9	1.7	7.3	(65)
29.5	8.8	----	----	---	26.2	46.2	3.7	2.8	7.8	(27)
----	8.6	----	----	---	30.4	52.5	---	---	---	(57)
31.2	---	----	----	---	----	----	3.7	0.9	6.3	(46)

[a]DM=dry matter, CP=crude protein, CF=crude fiber, NFE=nitrogen free extract, EE=ether extract, ADF=acid detergent fiber, NDF=neutral detergent fiber.

[b]High energy corn silage.

certain chemical fractions have been observed. Johnson and McClure (38) found digestibility of cellulose decreased from 73% in the blister stage to 55% for mature corn silage. Protein digestibility decreased from 73 to 64% for the above maturities. This is contrary to the findings of Owens et al. (65) who reported lower digestibility for high and low DM corn silage. However, Gordon et al. (27) found significantly higher acid detergent fiber digestibility and no difference in protein digestibility for normal and late harvested silages.

PREDICTING SILAGE QUALITY

Numerous chemical and biological methods have been suggested for predicting forage quality. The majority of these methods have been suggested and tested for perennial grasses and legumes and the efficacy of these procedures for predicting corn silage quality has not been adequately tested. Schmid et al. (70) published data on predicting digestibility of corn silage by biological methods, and Marten et al. (48) have reported on chemical methods for predicting digestibility. The biological methods tested by Schmid et al. (70) on 25 corn silages were: a modified Tilley and Terry two-stage fermentation, *in vitro* digestion of cell wall constituents, *in vitro* true DDM as described by Van Soest et

al. (79), direct acidification *in vitro* digestibility, six-hour *in vitro* fermentation, and cellulose digestibility. The modified Tilley and Terry procedure gave the highest correlation to *in vivo* DMD (coefficient of determination(R^2)=.70, S.E.=1.78). Multiple regression did not improve the prediction.

Marten et.al. (48) tested 19 laboratory assays for the prediction of corn silage *in vivo* DMD. The chemical methods included the Weende proximate analysis and Van Soest detergent analysis schemes with various modifications. From simple regression analysis, acid detergent fiber (ADF), cellulose, and crude fiber gave the highest R^2 of .61, .59, and .55, with standard error for the prediction ($S_{y.x}$) of 2.03, 2.06, and 2.18, respectively. Two variable models improved the coefficient of determination to .74 and .80 for the models including acid detergent lignin(ADL) plus ADL/ADF, and permanganate lignin(PL) plus PL/ADF, respectively. Marten et al. concluded that the added cost of employing an *in vitro* fermentation procedure relative to ADF, CF, and PL favors the use of these latter methods for routinely predicting digestibility (48).

NEAR INFRARED REFLECTANCE

Near-infrared reflectance (NIR) is a new methodology for forage quality evaluation which offers potential advantages over conventional laboratory analyses. The major advantage of infrared reflectance is the speed of analysis (8). A finely ground forage sample may be analyzed for several constituents in less than two minutes (11). The NIR technique was adapted for measuring oil, moisture, and protein content of grains and its major practical application presently is in marketing of wheat and soybeans. In 1976, Norris et al. (60) demonstrated that the NIR technique could be used for the evaluation of forages.

The IR technique of forage evaluation is strictly correlative, and therefore the accuracy of its predictions can never exceed the accuracy with which the measurement is calibrated (59). Spectral data is collected and processed by computer and predictions are made from multiple regression analysis. There are three keys to accurate prediction (72). First, the calibration samples must represent the population to be predicted and have accurate laboratory data. Second, the procedure must employ the mathematical treatment that will extract the maximum information from the spectral data. Third, the procedure requires applicable wavelengths. Wavelength selection is extremely difficult because it requires

greater computational capabilities than are available on commercial instruments (72).

Several companies manufacture IR instruments suitable for forage analysis. These include Dickey-John, Digilab, Lamont, Neotec, Nicolet, and Technicon (11).

Analysis of Forages by NIR

Norris et al. (59) designed and built a multipurpose computerized spectrophotometer with a Cary Model 14 monochromator operated in a single beam mode. Reflected radiation was collected with four lead-sulfide detectors. The signal from the detectors was amplified with a logarithmic-response amplifier, digitized and fed to a digital computer. The spectral reflectance curves were recorded as log(1/R) because this gives a curve comparable to an absorption curve with peak recordings occurring at wavelengths corresponding to absorption bands in the sample (60). Near infrared reflectance spectra (1.4 to 2.4 microns) were recorded for 87 samples of alfalfa, tall fescue, alfalfa bromegrass mixtures, and the tropical species bermudagrass and pangola digitgrass. Incorporating nine wavelengths for prediction equations, the correlation coefficients were .99 for crude protein (CP), .98 for neutral detergent fiber (NDF), .96 for acid detergent fiber (ADF), .96 for lignin (L), .95 for

IVDMD, .88 for DMD, .88 for DEI. Calibration equations generated from the odd-numbered samples predicted the values for the even numbered samples within a $S_{y.x}$ of $\pm$.95% for CP, $\pm$3.1% for NDF, $\pm$5.1% for DMD, and $\pm$7.9g for DMI. From the above results Norris et al. (60) concluded that IR has the potential for use in rapid evaluation of forage quality.

Shenk et al. (71) analyzed 38 forage concentrate diets by NIR. Coefficients of determination and S.E. of chemical constituents were .85 and .931 for CP, .96 and 1.880 for NDF, .98 and 1.148 for ADF, .94 and .459 for L, .95 and 1.401 for hemicellulose, .98 and .908 for cellulose, .48 and .018 for density, and .93 and 1.492 for IVDMD. Predictions were also made of weanling vole performance and the R^2 and the $S_{y.x}$ were .78, .23 for DMI (g/day), .94, 2.95 for DMD and .86, .11 for average daily gain (g/day). Sheep preference was measured on 15 crownvetch plots and predicted by NIR with an R^2 of .87 (71).

A few studies have been published employing the Neotec FQA51 Feed Quality Analyzer (3, 11, 56). Barton and Burdick (4) examined ten temperate and fourteen tropical grasses and concluded that the NIR spectrums for tropical and temperate species differed enough to warrant separate calibrations. Burdick et al. (11) predicted chemical composition of 11 bermudagrass samples and reported $S_{y.x}$ of 0.58, 1.07, 2.77, 0.66 and 1.90 for CP, ADF, NDF, L, and IVDMD, respectively.

From these results they concluded that near infrared reflectance is a useful tool for rapidly predicting forage quality. Moe and Carr (56) analyzed 27 rye silages by Neotec model FQA51 and reported an R^2 of .59 and $S_{y.x}$ of 6.7 for the prediction of IVDMD.

Chapter II

PROCEDURES

Sample Selection

A screening procedure was employed to obtain corn silage samples exhibiting deterioration and/or abnormal fermentations. Visual and olfactory characteristics of silage indicate possible fermentation types and the screening process was based on such characteristics. Samples were selected from corn silages submitted for analysis in the Virginia Tech Forage Testing Program. The screening procedure was employed to place silages into three broad classifications associated with known types of fermentation. The three types of fermentation were clostridial, normal (ie. lactic acid fermentation) and oxidized.

The classification of samples according to type of fermentation was determined by observation of color, aroma, and presence or absence of mold. Silages were selected exhibiting black, green-yellow, and brown colors. Five aromas were distinguished including: sour, pleasant, heated, no aroma (ie. absence of a distinctive or discernable aroma), and musty. The third characteristic in the screening process was the visible presence or absence of mold.

From observation of the above characteristics a composite evaluation of fermentation type was made. Considerable overlap of the classification characteristics occurred. The three characteristics of the sample were not always consistent with the classification. A silage might have a normal color but have a sour aroma indicating a clostridial fermentation. In some cases a silage was brown, mold was not present, and aroma was normal or could not be discerned and therefore, the sample was placed in the normal group.

Sample Preparation

Approximately 300g of wet material were obtained from corn silage samples selected for chemical analysis. Twenty-five grams of fresh silage was macerated with 125 ml of distilled water in a Waring Blender and filtered through four layers of cheesecloth. Silage pH was determined from the above extract with a Corning Model 7 pH meter. A 10 ml aliquot was frozen in 2 ml of 3 N sulfuric acid for lactate (2) determination. Duplicate 10 g samples of fresh silage were obtained for amyl alcohol plus xylene DM determination (74). Quadruplicate 5 g samples of fresh silage were taken for Kjeldahl nitrogen and volatile nitrogen analysis (1). The fresh silage for amyl alcohol plus xylene DM, Kjeldahl nitrogen, and volatile nitrogen were coarsely chopped in a

food processor. (La Machine manufactured by Moulinex Products Inc., Pennsauker, New Jersey). The remainder of the original 300 g sample (ca. 240 g) was dried in a forced air oven at 60° C for 48 hr. Samples were then ground to pass a 1mm screen and air equilibrated. Following air equilibration a 100° C oven DM was determined for correcting analyses to a DM basis.

Digestible Organic Matter

In vitro digestible organic matter (IVDOM) was determined by a method similar to the modification described by Barnes (6) of the Tilley and Terry procedure (77). The procedure differed from that described by Barnes in the following manner: Triplicate 400 mg air-equilibrated samples were weighed into 100 ml polypropylene centrifuge tubes. To each tube containing the sample was added 40 ml of inoculum composed of McDougall's buffer (55) and fresh rumen fluid in a 4:1 ratio. Included in each run were nine blank tubes containing inoculum without sample, and nine tubes containing a standard sorghum silage of known *in vivo* digestibility. Each tube was evacuated with carbon dioxide, fitted with a Bunsen valve, and placed in a 39° C water bath for 48 hr. After the 48 hr fermentation, 40 ml of an acid-pepsin solution was added to each tube before returning to the 39°

C water bath for an additional 48 hr digestion. After the second 48 hr period, contents of each tube were filtered through tared coarse porosity, sintered glass Gooch crucibles and dried in a 100° C oven. Digested residues and duplicate 1 g samples were determined for ash in a muffle furnace. In vitro digestible organic matter was determined by correcting IVDMD for ash content of the sample and the digested residue. IVDMD and IVDOM were calculated by the following formulas:

$$\text{IVDMD} = \frac{\text{sample DM} - \text{residue DM} - \text{blank DM}\ (100)}{\text{sample DM}}$$

$$\text{IVDOM} = \frac{\text{sample OM} - \text{residue OM} - \text{blank OM}\ (100)}{\text{sample OM}}$$

Fresh rumen fluid was obtained by vacuum pump from a mature, lactating Holstein cow fitted with rumen fistula. The donor cow was fed a corn silage ration containing 11% soybean meal on DM basis, 21 days prior to collecting fluid and throughout the collection period. The ration contained 16% CP on a DM basis. Rumen fluid was collected aproximately 90 minutes post feeding. Collections were made weekly on the same day each week.

Chemical Analysis

Acid detergent fiber and NDF were determined by the methods described by Goering and Van Soest (21). Chemically bound nitrogen was estimated by the acid detergent insoluble nitrogen (ADF-N) procedure suggested by Van Soest (78). Ammonia nitrogen content of the fresh sample was measured by distillation with carbonate-free magnesium oxide (1). Total nitrogen was determined by Kjeldahl procedure employing 4% boric acid for the nitrogen trap (1). Lactate content was determined from the frozen water extracts preserved in 3N sulfuric acid employing the colorimetric procedure of Barker and Summerson (2). Three separate DM determinations were made on the wet silage samples. Dry matter was estimated by drying at 85° and 60° C in a forced air oven for 24 and 48 hours, respectively. Dry matter was also measured by a two hour distillation of duplicate 10 g samples with xylene plus amyl alcohol in a 2:1 mixture (74). Potassium was measured by emission techniques employing a Perkin-Elmer Model 370 atomic absorption spectrophotometer.

Near-Infrared Reflectance (NIR)

Digestible organic matter of corn silage was predicted employing a Neotec Model FQA51 Feed Quality Analyzer. The FQA51 in combination with an Intel Model 8080 Micro-compu-

ter, employs six tilting filters and covers selected regions of the near infrared spectrum from 1.5 to 2.4 microns.

Air-equilibrated samples were ground in a cyclone sample mill for NIR analysis. The Versidump2 program which scans 89 pairs of wavelengths was applied to six corn silages which were representative of the four fermentation types. The change in optical density (delta OD) for each filter was obtained from the Versidump2 printout, and was used to determine pulse points and filters where changes in reflectance occurred. These areas of minimum and maximum reflectance were used for selecting pulse points which were then examined as possible areas in the near infrared spectrum where sample constituents related to digestibility could be measured.

A small calibration set of 10 silages which ranged from 38.5 to 74.7% IVDOM were selected from the corn silages utilized for chemical analysis. This small calibration set was used to test various combinations of pulse points as selected above. Multiple regression of optical data (delta OD) on IVDOM was performed by the Intel Model 8080 Micro-computer. The micro-computer employs stepwise regression techniques to select delta OD's which have a significant correlation to IVDOM. As many as six delta OD's may be selected as independent variables in the multiple regression equation. Com-

parison of correlation coefficients and $S_{y.x}$ for the prediction of the small calibration set determined the pulse points to be used for further analysis. Three pairs of pulse points were determined to give the best prediction of IVDOM based on the multiple correlation and standard error. The prediction was further tested by a 26 sample calibration set and the final prediction equation determined from this calibration. The prediction equation was tested by predicting IVDOM of 90 unknown corn silages. The 90 silages did not include samples which were previously used in either calibration set.

Statistical Analysis

Statistical analysis was done by simple and multiple linear regression, analysis of variance, and paired T-test procedures. All analysis was done using pre-programmed methods as outlined by the users guide to the Statistical Analysis System (SAS) 1979 (31).

Chapter III

RESULTS AND DISCUSSION

DATA DESCRIPTION

In vitro digestible organic matter was chosen as the index of feeding value to be predicted by laboratory analyses and infrared reflectance. IVDOM was preferred over IVDMD because IVDOM corrects for variation in digestible ash content of forages. This correction is important if soil contamination of the samples is a problem. This kind of improvement in laboratory analyses strengthens the NIR prediction (4). In grasses which have low fat content, DOM is virtually the same as TDN for which animal nutrient requirement tables are readily available (55). Limited observations of corn silage digestion studies at the Middleburg Forage Research Station indicate that DOM and TDN are essentially interchangable.

Mean IVDMD (67.7%) was 8% higher than IVDOM (59.8%) and the two measurements were highly correlated (r=.94). Larsen and Jones (43) reported IVDMD of 68.3% and IVDOM of 68.4% for 13 corn silages. Schmid et al. found IVDMD of 72% for 25 corn silages with a correlation to *in vivo* DMD of .83.

The overall means for the chemical constituents measured (Table 4) for the entire data set (142 silages) were

in agreement with published values for corn silage with some exceptions. Average lactate was considerably lower than has been reported elsewhere (Table 3) Lactate was low in most silages and was undetectable in 17 samples. The reason for the lower than normal lactate concentration is not readily apparent but probably is related to analysis conditions rather than limited fermentation of the silage. A possibility is that lactate was degraded to other metabolites during secondary fermentations either in the silo or in the sample during shipment to the laboratory. Kempton and Clemente (45) have shown that lactate can be replaced by butyrate in the silo, even after an original proliferation of lactic acid bacteria. In silages frozen shortly after sampling (standard fermentation group) mean lactate of 6.97% was consistent with published values (Table 3).

Secondary fermentations are an important consideration when relating laboratory measurements to feeding value. Readily degraded or volatile substances (eg. lactate, VFA's, WSC, NH_3/N) may provide indicators of fermentation type or forage quality under carefully controlled conditions but have limited value for practical forage testing applications. Further evidence that secondary fermentations occurred in these samples is that no detectable amounts of soluble carbohydrates were found for 30 silages analyzed.

Table 4. Chemical composition for entire data set of 142 corn silages.[a]

Measurement	Mean	S.D.	Range	Coefficient of variation
ADF (% of DM)	29.7	± 4.9	19.2 - 49.4	16.3
ADF-N (% of N)	15.4	± 6.6	7.5 - 42.2	42.5
ADF-N (% of DM)	0.2	± 0.1	0.1 - 0.8	47.0
CP (% of DM)	8.2	± 1.2	3.0 - 12.2	15.0
DM (%)	40.9	± 9.6	18.1 - 76.5	23.5
Lactate (% of DM)	1.7	± 2.3	0.0 - 11.7	138.9
NDF (% of DM)	60.3	± 7.7	39.5 - 82.6	12.7
pH	4.5	± 1.1	3.2 - 8.0	23.2
K (% of DM)	1.4	± 0.5	0.3 - 4.3	36.0
NH_3/N (% of N)	8.9	± 4.5	0.9 - 36.9	51.0
NH_3/N (% of DM)	0.1	± 0.1	0.0 - 0.7	62.5
IVDOM (%)	59.8	± 6.6	38.5 - 74.7	11.0
IVDMD (%)	67.7	± 6.5	48.7 - 84.0	9.6

[a]ADF=acid detergent fiber, ADF-N=acid detergent fiber nitrogen, CP=crude protein, DM=dry matter, NDF= neutral detergent fiber, K=potassium, NH_3/N=ammonia nitrogen.

An extremely wide range was obtained for all measurements. This variation was expected because the screening method was designed to select abnormal and deteriorated silages. The screening method probably biased the data set but at the same time resulted in a better understanding of the variability possible in corn silage.

Means by fermentation group are in Table 5. The standard group provides a comparison of normal silages to the other fermentation groups. The standard group was comprised of silages from the Virginia Tech Dairy Center and the Middleburg Forage Research Station. These silages were considered normal in that they supported normal growth rates for beef heifers and above average milk production. Silage samples from this group were better protected from secondary fermentations than were forage testing samples. Standard samples were either frozen shortly after collection or analyses (ie. NH_3/N, CP, DM) were determined on the fresh sample.

Higher quality silage is indicated in the standard group by significantly higher IVDOM and IVDMD than the selected groups. The selected groups (normal, clostridial, oxidized) were not significantly different in digestibility. Higher digestibility for the standard group indicates well preserved silage made from excellent corn and these samples

Table 5. Means, standard deviations, and ranges of chemical assays by fermentation group.

Constituent[1]	Standard (N=19)	Normal (N=19)	Clostridial (N=49)	Oxidized (N=55)
ADF	23.06[a]	29.82[b]	31.14[b]	31.41[b]
(% of DM)	1.77	4.16	3.94	4.55
	19.50-25.90	19.18-41.18	27.04-42.02	23.18-49.40
ADF-N	20.08[a]	12.63[b]	14.41[bc]	16.71[c]
(% of N)	9.10	4.43	3.02	6.90
	9.24-36.01	7.51-30.04	8.56-19.62	8.16-42.19
ADF-N	0.28[a]	0.16[b]	0.20[bc]	0.22[c]
(% of DM)	0.14	0.05	0.05	0.10
	0.13-0.51	0.11-0.34	0.12-0.36	0.09-0.77
CP	8.59	8.05	8.57	8.17
(% of DM)	1.05	0.92	1.03	1.54
	6.39-10.41	5.63-10.31	7.05-12.16	3.01-12.00
DM (%)	40.29[a]	38.25[a]	34.45[a]	45.57[b]
	4.48	9.99	6.31	9.42
	33.80-50.00	18.10-73.90	24.50-45.60	27.20-76.50
Lactate	6.97[a]	1.33[b]	0.69[c]	0.53[c]
(% of DM)	1.89	0.98	0.78	0.67
	4.03-11.67	0.00-4.40	0.00-2.71	0.00-3.63
NDF	59.51	57.51	60.01	63.14
(% of DM)	6.28	7.11	5.44	8.39
	50.78-70.14	39.51-75.78	49.15-71.00	41.16-82.60
pH	3.89[a]	4.15[a]	5.00[b]	4.93[b]
	0.17	0.44	1.30	1.28
	3.65-4.40	3.20-6.07	3.72-7.95	3.79-7.89
K	1.75[a]	1.30[b]	1.39[b]	1.29[b]
(% of DM)	0.70	0.28	0.34	0.55
	1.19-3.77	0.65-1.83	0.92-2.10	0.27-4.33
NH_3/N	7.42[a]	9.40[ab]	10.32[b]	8.43[ab]
(% of N)	2.52	2.84	7.92	4.63
	4.21-12.98	2.17-15.97	0.97-36.92	0.86-17.51
NH_3/N	0.09[a]	0.11[ab]	0.14[b]	0.10[a]
(% of DM)	0.03	0.03	0.14	0.06
	0.06-0.15	0.03-0.21	0.01-0.66	0.01-0.24
IVDOM (%)	63.95[a]	60.14[b]	59.12[b]	58.21[b]
	2.02	6.76	5.25	7.22
	60.99-67.90	43.02-72.73	48.47-68.68	38.49-74.73
IVDMD (%)	72.90[a]	68.03[b]	67.29[b]	65.70[b]
	3.05	7.05	5.53	6.16
	67.37-79.04	53.52-84.00	55.34-77.06	48.71-79.85

[a,b,c]Means within a given row not bearing a common superscript differ ($P < .05$).

[1]For explanation of abreviations see Table 4.

were better protected than the forage testing samples which were exposed to unfavorable conditions. Forage testing samples are exposed to aerobic conditions for various periods of time. Samples may spend several days in the mail before reaching the laboratory. Forage testing samples were analyzed in this study because such samples are exposed to conditions expected to occur in a practical forage analysis situation.

Standard silages were significantly lower in ADF which is consistent with digestibility results. ADF is negatively correlated with digestibility and would be expected to be lower in higher quality silage (48). Contrary to the above results, ADF-N was significantly higher for the standard group. Because ADF-N represents relative percentage of bound nitrogen in a forage, higher levels of ADF-N would be expected to correspond to lower quality silage. This apparent discrepancy between chemical analysis and theory is probably an artifact. The samples from the standard group were difficult to filter. Filtering problems can lead to incomplete washing of the digested residues resulting in detergent residue and free nitrogen appearing in the ADF-N fraction (4, 48).

Lactate was significantly higher in the standard group and was near levels reported elsewhere (Table 3). As men-

tioned previously, a large proportion of this difference in lactate could be due to protection of the samples from secondary fermentations. Differences in extent of fermentation are indicated by significantly higher lactate for the normal group versus clostridial and oxidized. However, the mean of 1.33% is substantially lower than what would be expected for normal corn silage (Table 5). Standard and normal silages were not different in pH but were significantly lower than clostridial and oxidized silages. Values for the former groups were comparable to published results (Table 3). The lower pH for the standard and normal silages was also consistent with results for lactate.

Crude protein values were comparable to published levels (Table 3). There were no differences among the four fermentation groups for NDF and CP. Ammonia nitrogen levels were significantly higher for the clostridial group which may correspond to amino acid catabolism by Clostridia. The volatile nature of ammonia makes interpretation of data from these samples questionable since it is not known if NH_3/N was lost to the environment before analysis. One would expect a lower nitrogen content if NH_3/N were lost to the atmosphere. At pH 3.7 to 4.0 considerable NH_3/N would exist as the NH_4 ion and would be recovered.

Standard silages were significantly higher than the other groups in potassium. Potassium levels are substantially higher in the corn plant relative to the grain. Higher potassium levels in the standard group would indicate lower grain content and more immature silages. However, potassium is also related to level of fertilization. Since the standard group represents silages from only two locations, the higher potassium content may indicate fertility at those locations.

Mean chemical composition by color, aroma, and mold are in Table 6, Table 7, and Table 8, respectively. Green-yellow silages had significantly lower ADF (28.35%) than either brown (30.26%) or black (35.70%). The pleasant smelling silages were significantly lower in ADF than other aromas (sour, heated, no aroma, musty). Sour and musty silages showed higher pH than the other aromas. Moldy silages were higher in DM and pH than silages not exhibiting mold. However, these differences in chemical composition were not reflected in differences in IVDOM among levels of color, aroma, and mold groups.

Table 9 shows the correlations among the chemical constituents and *in vitro* digestibility. Nitrogen, fermentation products, and plant fiber components had low correlations with IVDOM. The low correlations may be a result of

Table 6. Means, standard deviations, and ranges of chemical assays grouped by color.

Constituent[1]	Black (N=8)	Green-yellow (N=64)	Brown (N=70)
ADF (% of DM)	35.70[a] 6.93 27.04-49.40	28.35[b] 4.88 19.50-41.18	30.26[c] 3.91 19.18-42.02
ADF-N (% of N)	22.43[a] 8.46 11.62-40.45	15.03[b] 7.00 7.51-36.01	15.03[b] 5.49 7.97-42.19
ADF-N (% of DM)	0.35[a] 0.19 0.14-0.77	0.20[b] 0.10 0.11-0.51	0.19[b] 0.05 0.09-0.35
CP (% of DM)	9.26[a] 2.06 7.05-12.16	8.30[b] 0.93 6.39-10.41	8.06[b] 1.32 3.01-12.00
DM (%)	42.48[ab] 7.92 25.30-51.70	37.12[a] 7.74 18.10-73.90	44.08[b] 10.17 24.50-76.50
Lactate (% of DM)	0.29[a] 0.27 0.00-0.64	2.97[b] 2.94 0.00-11.67	0.68[a] 0.75 0.00-3.63
NDF (% of DM)	62.24 6.94 54.83-71.73	59.01 6.90 39.51-75.78	61.25 8.31 41.16-82.60
pH	5.58[a] 1.67 4.09-7.95	4.16[b] 0.59 3.20-7.81	4.76[c] 1.16 3.72-7.89
K (% of DM)	1.72[a] 1.11 1.02-4.33	1.46[a] 0.48 0.65-3.77	1.24[b] 0.35 0.27-2.50
NH_3/N (% of N)	11.51 11.47 1.60-36.92	8.30 3.11 2.17-15.97	9.11 4.30 0.86-17.51
NH_3/N	0.17[a] 0.21 0.02-0.66	0.10[b] 0.04 0.03-0.21	0.11[b] 0.06 0.01-0.24
IVDOM (%)	55.77 9.43 38.49-65.03	60.19 5.95 43.02-72.23	59.84 6.67 39.04-74.73
IVDMD (%)	63.74[a] 8.47 48.71-72.24	68.52[ab] 6.71 53.52-84.00	67.36[b] 5.88 53.88-79.85

[a,b,c]Means within a given row not bearing a common superscript differ ($P < .05$).

[1]For explanation of abreviations see Table 4.

Table 7. Means, standard deviations, and ranges of chemical assays grouped by aroma.

Constituent[1]	Sour (N=20)	Pleasant (N=62)	Heated (N=32)	No aroma (N=10)	Musty (N=18)
ADF	31.14[a]	27.79[b]	31.85[a]	30.65[a]	30.39[a]
(% of DM)	3.83	4.84	5.25	3.57	3.50
	27.04-42.02	19.18-41.18	23.18-49.40	25.36-36.74	24.76-37.90
ADF-N	14.36	14.54	17.69	15.40	15.83
(% of N)	2.95	6.93	7.36	6.34	6.44
	8.56-19.62	7.51-36.01	9.38-42.19	8.79-29.45	8.16-31.73
ADF-N	0.20	0.19	0.23	0.19	0.20
(% of DM)	0.05	0.10	0.11	0.08	0.08
	0.12-0.36	0.11-0.51	0.14-0.77	0.09-0.34	0.12-0.42
CP	8.52	8.26	8.39	7.72	7.87
(% of DM)	1.04	0.96	1.75	1.09	1.22
	7.05-12.16	6.39-10.41	3.01-12.00	5.63-9.13	5.94-10.93
DM (%)	34.41	38.95	44.25	42.58	47.56
	6.15	9.06	10.46	7.29	8.20
	24.50-45.60	18.10-73.90	27.20-76.50	31.90-53.90	39.00-66.70
Lactate	0.65	3.03	0.59	1.17	0.44
(% of DM)	0.78	2.94	0.66	1.08	0.75
	0.00-2.71	0.00-11.67	0.00-3.63	0.00-2.81	0.00-2.94
NDF	60.06	58.06	62.31	59.43	65.13
(% of DM)	5.30	7.11	9.13	4.11	7.98
	49.15-71.00	39.51-75.78	41.16-82.60	52.15-63.84	53.49-80.67
pH	4.97[a]	4.07[b]	4.35[b]	4.14[b]	6.19[c]
	1.27	0.41	0.57	0.12	1.46
	3.72-7.95	3.20-6.07	3.79-6.61	4.01-4.39	4.12-7.89
K	1.38	1.45	1.35	1.20	1.20
(% of DM)	0.33	0.49	0.67	0.27	0.29
	0.92-2.10	0.65-3.77	0.27-4.33	0.61-1.43	0.78-2.06
NH_3/N	10.17	8.88	9.19	9.78	6.41
(% of N)	7.74	2.97	4.09	2.43	5.37
	0.97-36.92	2.17-15.97	1.60-16.96	6.24-14.19	0.86-17.51
NH_3/N	0.14[a]	0.11[a]	0.12[a]	0.11[a]	0.06[b]
(% of DM)	0.14	0.03	0.06	0.04	0.05
	0.01-0.66	0.03-0.21	0.02-0.24	0.08-0.19	0.01-0.19
IVDOM (%)	59.05	61.17	56.60	61.20	60.54
	5.12	6.19	8.18	4.62	5.26
	48.47-68.68	43.02-72.73	38.49-74.73	53.40-66.47	51.71-70.86
IVDMD (%)	67.33[ab]	69.43[a]	64.31[b]	68.89[ab]	67.36[ab]
	5.38	6.72	6.84	3.84	5.08
	55.34-77.06	53.52-84.00	48.71-79.85	60.45-73.50	60.30-77.90

[a,b,c]Means within a given row not bearing a common superscript differ ($P < .05$).

[1]For explanation of abreviations see Table 4.

Table 8. Means, standard deviations, and ranges of chemical assays grouped by mold.

Constituent[1]	Positive (N=27)	Negative (N=115)
ADF	30.87	29.43
(% of DM)	3.86	5.03
	24.76-38.96	19.18-49.40
ADF-N	15.22	15.50
(% of N)	5.02	6.89
	8.16-24.12	7.51-42.19
ADF-N	0.20	0.20
(% of DM)	0.08	0.10
	0.11-0.42	0.10-0.77
CP	8.21	8.24
(% of DM)	1.41	1.20
	5.94-12.16	3.01-12.00
DM (%)	45.59[a]	39.74[a]
	8.81	9.47
	30.50-66.70	18.10-76.50
Lactate	0.45[a]	1.98[b]
(% of DM)	0.65	2.50
	0.00-2.94	0.00-11.67
NDF	63.26	59.60
(% of DM)	8.70	7.28
	42.20-80.67	39.51-82.60
pH	5.60[a]	4.28[b]
	1.47	0.74
	3.82-7.95	3.20-7.81
K	1.20[a]	1.41[b]
(% of DM)	0.26	0.52
	0.78-2.06	0.27-4.33
NH_3/N	7.47	9.21
(% of N)	7.19	3.60
	0.86-36.92	0.97-17.51
NH_3/N	0.10	0.11
(% of DM)	0.12	0.05
	0.01-0.66	0.01-0.24
IVDOM (%)	59.42	59.85
	5.68	6.76
	46.60-70.86	38.49-74.73
IVDMD (%)	66.71	67.91
	5.55	6.67
	53.88-77.90	48.71-84.00

[a,b]Means within a given row not bearing a common superscript differ ($P < .05$).

[1]For explanation of abreviations see Table 4.

the abnormal fermentations in the data set. As expected, ADF and ADF-N were negatively correlated with IVDOM. Crude protein was positively correlated with IVDMD and negatively correlated to IVDOM. Marten et al. (48) found CP was negatively correlated to in vivo DMD. Theoretically the correlation of CP and digestibility should be positive. However, elevated protein values in silages suggest an immature plant or high plant:grain ratio. Extensive deterioration or microbial degradation in the silo would reverse the expected higher protein-higher digestibility relationship. The actual correlation of -.029 is small, and indicates that CP is not indicative of changes in fermentation that are predictable.

Potassium had a negative correlation with digestibility. Since the level of potassium is substantially higher in the plant relative to the grain fraction of the silage, lower potassium content may correspond to higher grain content of the silage. This would exlpain the relationship of potassium to digestibility. Weaver et al. (82) has shown that increasing grain content will increase digestibility until the grain has reached physiological maturity. Dry matter had a strong positive correlation to digestibility. The data does not indicate why DM has a strong correlation to IVDOM but DM may be related to maturity (27, 35, 38) and

type of fermentation. Apparently high DM is not as detrimental to preservation and fermentation characteristics as low DM (44). This creates a confounding influence of DM in that high DM silages may be either high quality, or severely deteriorated (ie. oxidized or moldy).

It should be recognized, that IVDOM is a closed system and a 48 hr fermentation should be an essentially complete digestion. A shorter fermentation period might show larger differences in the rate of digestion among different fermentation types.

SIMPLE LINEAR REGRESSION

IVDOM was regressed on the eleven chemical constituents. Regression models, coefficients of determination, and standard errors for the prediction are in Table 10. The standard error for the prediction ($S_{y.x}$) is defined as the mean square error of the regression.

Magnitude of R^2 were not large. The ranking of individual measurements by R^2 was similar to the findings of Marten et al. (48). The best predictor of IVDOM was ADF which explained 35.2% of the variation with $S_{y.x}$ of 5.29. Marten et al. (48) found ADF was the best predictor of *in vivo* DMD, accounting for 61% of the variation. From Table 10, DM explained 20.8% of the variation in IVDOM with $S_{y.x}$ of 5.86.

Table 9. Correlations among chemical constituents, IVDMD and IVDOM for 142 corn silages.[1]

	ADF (% of DM)	ADF-N (% of N)	ADF-N (% of DM)	CP (% of DM)	DM(%)	Lactate (% of DM)	NDF (% of DM)	pH	K (% of DM)	NH_3/N (% of N)	NH_3/N (% of DM)	IVDMD(%)	IVDOM(%)
ADF (% of DM)	---	0.189	0.154	-0.079	-0.285	-0.090	0.213	0.102	0.028	0.039	0.047	-0.638	-0.594
ADF-N (% of N)	0.189	---	0.871	-0.071	-0.042	-0.187	-0.005	0.052	0.095	-0.249	-0.168	-0.210	-0.180
ADF-N (% of DM)	0.154	0.871	---	0.369	0.008	-0.164	-0.073	0.054	0.317	-0.119	0.039	-0.095	-0.176
CP (% of DM)	-0.079	-0.071	0.369	---	0.047	-0.004	-0.153	0.024	0.367	0.190	0.417	0.023	-0.029
DM (%)	-0.285	-0.042	0.008	0.047	---	-0.100	0.064	0.247	-0.187	-0.067	-0.048	0.402	0.456
Lactate (% of DM)	-0.090	-0.187	-0.164	-0.004	-0.100	---	-0.327	-0.307	0.019	-0.151	0.102	0.168	0.126
NDF (% of DM)	0.213	-0.005	-0.073	-0.153	0.064	-0.327	---	0.217	0.019	-0.017	-0.069	-0.249	-0.159
pH	0.102	0.052	0.054	0.024	0.247	-0.307	0.217	---	-0.120	-0.083	-0.060	-0.011	0.045
K (% of DM)	0.028	0.095	0.317	0.367	-0.187	0.019	0.019	-0.120	---	0.083	0.120	-0.081	-0.162
NH_3/N (% of N)	0.039	-0.249	-0.119	0.190	-0.067	0.151	-0.017	-0.083	0.083	---	0.911	-0.082	-0.057
NH_3/N (% of DM)	0.047	-0.168	0.039	0.417	-0.048	0.102	-0.069	-0.060	0.120	0.911	---	-0.087	-0.081
IVDMD (%)	-0.638	-0.120	-0.095	0.023	0.402	0.168	-0.249	-0.011	-0.081	-0.082	-0.087	---	0.945
IVDOM (%)	-0.594	-0.180	-0.176	-0.029	0.456	0.126	-0.159	0.045	-0.162	-0.057	-0.081	0.945	---

[1] For explanation of abreviations see Table 4.

Table 10. Simple linear regression of IVDOM on laboratory measurements for full data set of 142 corn silages.

Constituent[1]	a	b	R^2	$S_{y \cdot x}$
ADF (% of DM)	83.585	-0.802[a]	.352	5.29
ADF-N (% of N)	62.550	-0.180[a]	.033	6.47
ADF-N (% of DM)	62.229	-12.152[a]	.031	6.47
CP (% of DM)	61.049	-0.156	.001	6.58
DM (%)	47.057	0.311[a]	.208	5.86
Lactate (% of DM)	58.930	0.986	.016	6.53
NDF (% of DM)	67.973	-0.136	.025	6.49
pH	58.488	0.282	.002	6.57
K (% of DM)	62.713	-2.157	.026	6.49
NH_3/N (% of N)	60.501	-0.083	.003	6.57
NH_3/N (% of DM)	60.615	-7.842	.007	6.56

[a]Regression coefficient is significant ($P < .05$).

[b]For explanation of abreviations see Table 4.

The correlation between DM and ADF was significant r = 0.285. None of the other constituents explained more than 5% of the variation in IVDOM.

Regression analysis was also performed within fermentation groups to determine if different models would be appropriate for different types of fermentations. Variables of minor importance when considering the entire data set became the best predictors of IVDOM when only one fermentation type was analyzed.

For the standard group (Table 11) ADF and DM had the highest R^2, respectively. Potassium explained 10.4% of the variation in IVDOM. The positive correlation for potassium for the standard group would suggest that for this group potassium may be related to maturity of the silage.

Within the clostridial group (Table 12) potassium was the best predictor explaining 34.0% of the variation in IVDOM with a $S_{y.x}$ of 4.39. Potassium levels are substantially higher in the corn plant compared to the corn grain. As the grain fraction of the silage increased relative to the plant, digestibility of the silage would be expected to increase and percent potassium decrease. This theoretical relationship is consistent with the negative slope of the regression line obtained. However, clostridial silages are normally lower DM and more immature and potassium is known

Table 11. Simple linear regression of IVDOM on laboratory measurements for 19 corn silages in standard group.

Constituent[1]	a	b	R^2	$S_{y.x}$
ADF (% of DM)	78.163	-0.616	.290	1.75
ADF-N (% of N)	63.640	0.015	.005	2.08
ADF-N (% of DM)	63.634	1.126	.006	2.08
CP (% of DM)	60.655	0.384	.040	2.04
DM (%)	56.327	0.189	.175	1.89
Lactate (% of DM)	62.519	2.070	.038	2.04
NDF (% of DM)	62.375	0.026	.007	2.07
pH	51.583	3.175	.067	2.01
K (% of DM)	62.313	0.935	.104	1.97
NH_3/N (% of N)	64.757	-0.109	.018	2.06
NH_3/N (% of DM)	64.396	-4.873	.004	2.08

[a]Regression coefficient is significant ($P < .05$).

[b]For explanation of abreviations see Table 4.

Table 12. Simple linear regression of IVDOM on laboratory measurements for 19 corn silages in clostridial group.

Constituent[1]	a	b	R^2	$S_{y \cdot x}$
ADF (% of DM)	72.075	-0.416	.097	5.14
ADF-N (% of N)	53.829	0.367	.045	5.28
ADF-N (% of DM)	56.206	14.683	.022	5.35
CP (% of DM)	60.748	-0.190	.001	5.40
DM (%)	46.794	0.358	.185	4.88
Lactate (% of DM)	59.304	-0.272	.002	5.40
NDF (% of DM)	78.458	-0.322	.111	5.10
pH	51.861	1.452	.129	5.04
K (% of DM)	71.801	-9.142[a]	.340	4.39
NH_3/N (% of N)	59.060	0.005	.000	5.41
NH_3/N (% of DM)	58.806	2.235	.003	5.40

[a]Regression coefficient is significant ($P < .05$)

[b]For explanation of abreviations see Table 4.

to be related to maturity of grasses. Therefore, if grain content were constant, potassium might indicate maturity of the plant.

Dry matter and pH were the second and third best predictors of IVDOM. Dry matter was positively correlated and explained 18.5% of the variation. Unless considered only within known fermentation groups, DM is a questionable predictor of digestibility. Within the clostridial group pH was positively correlated and explained 12.9% of of the variation but no relationship to IVDOM was found when considering the full data set. This could be explained by the fact that pH is a critical indicator of clostridial activity but the relationship is not consistant when considering corn silage within the normal pH range of 3.7 to 4.0.

Within the normal group (Table 13) DM had the largest R^2 (.430) and lowest $S_{y.x}$ (5.28). ADF explained a significant proportion of the variation in IVDOM with an R^2 of .378 and $S_{y.x}$ of 5.39. The above were the only variables which explained a significant fraction of the variation within the normal group.

These results emphasize that the standard and normal groups were composed of considerably different types of silages. The largest difference may have been the degree of protection from secondary fermentations.

Table 13. Simple linear regression of IVDOM on laboratory measurements for 49 corn silages in normal group.

Constituent[1]	a	b	R^2	$S_{y \cdot x}$
ADF (% of DM)	89.924	-0.999[a]	.378	5.39
ADF-N (% of N)	64.091	-0.313	.042	6.69
ADF-N (% of DM	65.870	-35.883	.063	6.61
CP (% of DM)	65.179	-0.626	.007	6.81
DM (%)	43.690	0.430[a]	.403	5.28
Lactate (% of DM)	59.024	0.839	.015	6.78
NDF (% of DM)	73.569	-0.234	.060	6.62
pH	59.898	0.057	.000	6.83
K (% of DM)	65.702	-4.286	.032	6.72
NH_3/N (% of N)	61.774	-0.174	.005	6.81
NH_3/N (% of DM	62.587	-22.029	.013	6.79

[a]Regression coefficient is significant ($P < .05$)

[b]For explanation of abreviations see Table 4.

The oxidized group (Table 14) included not only silages that had obviously been oxidized but also any silage which appeared to have been exposed to aerobic conditions (eg. moldy). Many of these samples did not exhibit the characteristic burned or caramelized aroma of heated silages. Dry matter was positively correlated and had the largest R^2 .320 with a $S_{y.x}$ of 6.01. Acid detergent fiber was negatively correlated and was the second best predictor of IVDOM with an R^2 of .314 and $S_{y.x}$ of 6.04. It is worth noting that ADF-N expressed as a percentage of the DM was negatively correlated, and explained 19.2% of the variation in IVDOM for the oxidized group. This would indicate that a negative relationship between bound nitrogen and digestibility does exist for corn silage. Potassium was negatively correlated and made a significant contribution to explaining IVDOM with an R^2 of .151 and $S_{y.x}$ of 6.71.

These results indicate that ADF and DM are consistently related to IVDOM, however, other measurements might be useful predictors within a given fermentation type. According to Van Soest (80) fiber represents the least digestible portion of the forage and although ADF does not represent a chemically uniform substance, it does represent a fraction of lower digestibility. Therefore, one could expect ADF to have a significant negative correlation to digestibility as

Table 14. Simple linear regression of IVDOM on laboratory measurements for 55 corn silages in oxidized group.

Constituent[1]	a	b	R^2	$S_{y \cdot x}$
ADF (% of DM)	86.148	-0.889[a]	.314	6.04
ADF-N (% of N)	65.396	-0.430[a]	.169	6.64
ADF-N (% of DM)	64.997	-31.475[a]	.192	6.55
CP (% of DM)	60.639	-0.297	.004	7.27
DM (%)	38.468	0.433[a]	.320	6.01
Lactate (% of DM)	57.426	1.483	.019	7.22
NDF (% of DM)	59.205	-0.016	.000	7.29
pH	52.900	1.077	.036	7.15
K (% of DM)	64.813	-5.127[a]	.151	6.71
NH_3/N (% of N)	58.791	-0.068	.002	7.28
NH_3/N (% of DM)	59.942	-17.139	.021	7.21

[a]Regression coefficient is significant ($P < .05$).

[b]For explanation of abreviations see Table 4.

these data indicates. The relationship of DM to digestibility is not as readily apparent. Dry matter content of corn silage increases with maturity and with increasing grain:plant ratio (25, 27, 38). Digestibility of corn silage tends to increase and then level off during the milk, early dough, and dough dent stages of maturity (38). Therefore, increased DM could indicate later maturity and higher digestibility. Dry matter affects extent of fermentation and DM tends to increase under aerobic conditions (27, 44). Therefore, DM might be related to abnormal fermentations. The data shows that DM was not significant for the regression within the clostridial group which is composed of relatively low DM silages or the standard group which did not contain abnormal silages. Niether theory nor the data from this study show a clear cause and effect relationship between DM and digestibility.

Regression analysis within the fermentation groups seems to suggest adopting more than one equation for corn silage feeding value. However, the development of a quantitative method for indexing silages by type of fermentation may be impractical.

MULTIPLE REGRESSION

Multiple regression models were selected by largest R^2 from all possible regressions and also forward stepwise regression with maximum R^2 improvement. Analysis was performed on the entire data set and the four fermentation groups seperately.

Including ADF and DM, the two variable model employing the full data set (Table 15) explained 44.2% of the variation in IVDOM with a $S_{y.x}$ of 4.93. There was little improvement in R^2 by adding more variables and only ADF and DM had significant regression coefficients for all eleven models (Table 15). The full model explained 47.6% of the variation in IVDOM with a $S_{y.x}$ of 4.94.

Within the standard group (Table 16) ADF and ADF-N explained 35.4% of the variation in IVDOM with a $S_{y.x}$ of 1.72. In the one, two, and three variable models ADF had the only significant regression coefficient. Suprisingly, the inclusion of CP in the best four variable model increased the R^2 to .513 and the regression coefficients for ADF, CP, NH_3/N(% of N), and NH_3/N(% of DM) became significant. Adding potassium to the five variable model further increased R^2 to .723 and reduced the $S_{y.x}$ to 1.25. Addition of the other variables resulted in small R^2 improvements to .806, and increased the $S_{y.x}$ to 1.43 for the full model.

Table 15. Multiple regression of IVDOM on laboratory measurements for 142 corn silages.[1]

Parameter	Number of variables in model										
	1	2	3	4	5	6	7	8	9	10	11
	regression coefficients										
Intercept Y	83.585	71.323	69.524	71.822	71.793	74.117	76.915	76.949	76.662	77.964	77.958
ADF (% of DM)	-0.802[a]	-0.682[a]	-0.662[a]	-0.665[a]	-0.651[a]	-0.632[a]	-0.648[a]	-0.650[a]	-0.645[a]	-0.648[a]	-0.648[a]
ADF-N (% of N)							-0.069	-0.062	-0.067	-0.123	-0.123
ADF-N (% of DM)					-4.467	-5.236	-0.531			4.368	4.364
CP (% of DM)								-0.426	-0.351	-0.480	-0.480
DM (%)		0.213[a]	0.224[a]	0.211[a]	0.216[a]	0.220[a]	0.217[a]	0.211[a]	0.211[a]	0.208[a]	0.208[a]
Lactate (% of DM)			0.898	0.896	0.822	0.678	0.763	0.777	0.795	0.795	0.795
NDF (% of DM)						-0.048	-0.065	-0.059	-0.059	-0.058	-0.058
pH							0.424	0.393	0.382	0.383	0.383
K (% of DM)				-1.234	-0.945	-0.867	-0.424	-0.645	-0.665	-0.749	-0.749
NH_3/N (% of N)											0.001
NH_3/N (% of DM)									-3.160	-3.256	-3.290
R^2	0.352	0.442	0.455	0.463	0.466	0.469	0.473	0.475	0.475	0.476	0.476
$S_{y \cdot x}$	5.29	4.93	4.89	4.87	4.88	4.88	4.88	4.89	4.91	4.92	4.94

[a] Regression coefficient is significant ($P < .05$).

[1] For explanation of abreviations see Table 4.

Table 16. Multiple regression of IVDOM on laboratory measurements for 19 corn silages in standard group.[1]

Parameter	Number of variables in model										
	1	2	3	4	5	6	7	8	9	10	11
	regression coefficients										
Intercept Y	78.163	79.210	75.765	121.312	129.641	130.592	116.956	116.947	103.726	92.323	91.986
ADF (% of DM)	-0.616[a]	-0.714[a]	-0.645[a]	-1.071[a]	-1.029[a]	-0.983[a]	-0.867[a]	-0.830[a]	-0.791[a]	-0.691	-0.690
ADF-N (% of N)		0.059	0.080						0.360	0.545	0.544
ADF-N (% of DM)								-1.540	-25.754	-38.147	-38.044
CP (% of DM)				-3.844[a]	-5.341[a]	-5.652[a]	-5.692[a]	-5.762[a]	-4.521	-3.692	-3.674
DM (%)										0.058	0.056
Lactate (% of DM)						2.716	4.336	4.938	4.358	4.499	4.452
NDF (% of DM)											0.009
pH							2.845	3.194	3.207	3.018	3.009
K (% of DM)			0.831		1.697[a]	1.544[a]	1.543[a]	1.503[a]	1.549[a]	1.400	1.400
NH_3/N (% of N)				-3.970[a]	-5.902[a]	-6.236[a]	-6.236[a]	-6.410[a]	-5.756[a]	-5.214[a]	-5.207
NH_3/N (% of DM)				325.240[a]	488.263[a]	504.966[a]	495.399[a]	503.578[a]	456.188[a]	415.689[a]	414.965[a]
R^2	0.290	0.354	0.421	0.513	0.723	0.744	0.783	0.786	0.800	0.806	0.806
$S_{y \cdot x}$	1.75	1.72	1.69	1.60	1.25	1.25	1.21	1.26	1.28	1.34	1.43

[a]Regression coefficient is significant ($P < .05$).

[1]For explanation of abreviations see Table 4.

For the normal group (Table 17) DM was the most effective predictor of IVDOM. The second variable added was ADF and this model explained 50.3% of the variation with $S_{y.x}$ of 4.87. There were minor R^2 improvements after two variables. DM and ADF were significant in all models and slightly higher R^2 (.553) was achieved with the normal group versus the full data set R^2 (.476).

The clostridial group (Table 18) differed in that potassium was the most effective predictor in the one variable model and the second variable added was ADF-N (% of N). The model including potassium and ADF-N (% of N) had an R^2 of .488 and $S_{y.x}$ of 3.99. ADF was the third variable added but was not significant in any of the models. The full model explained 67.5% of the variation with $S_{y.x}$ of 4.80.

The oxidized group (Table 19) followed a similar pattern to the full data set with ADF and DM the first and second variables included. The two variable model with ADF and DM explained 48.4% of the variation with $S_{y.x}$ of 5.28. There was improvement in R^2 of approximately 10% with additional variables. ADF and DM were the only variables significant in any of the models. The full model explained 57.8% of the variation in IVDOM with $S_{y.x}$ of 5.26.

Coefficients of determination and $S_{y.x}$ were similar among the fermentation groups except for the standard group.

Table 17. Multiple regression of IVDOM on laboratory measurements for 49 corn silages in normal group.[1]

Parameter	Number of variables in model										
	1	2	3	4	5	6	7	8	9	10	11
	regression coefficients										
Intercept Y	43.690	67.499	81.772	80.363	77.580	77.397	98.466	94.149	105.926	103.656	103.576
ADF (% of DM)		-0.616[a]	-0.720[a]	-0.713[a]	-0.752[a]	-0.706[a]	-0.682[a]	-0.712[a]	-0.737[a]	-0.746[a]	-0.747[a]
ADF-N (% of N)						-0.133	-0.129	-0.171	-0.942	-0.834	-0.840
ADF-N (% of DM)									66.148	57.099	57.555
CP (%of DM)			-1.250	-1.194	-1.168	-1.365	-3.361	-3.279	-4.719	-4.622	-4.634
DM (%)	0.430[a]	0.288[a]	0.258[a]	0.258[a]	0.258[a]	0.263[a]	0.260[a]	0.261[a]	0.255[a]	0.261[a]	0.260[a]
Lactate (% of DM)				0.567	0.799	0.989	0.909	1.243	1.213	1.215	1.217
NDF (% of DM)					0.061	0.088		0.082	0.081	0.090	0.091
pH											0.039
K (% of DM)										0.750	0.746
NH_3/N (% of N)							-1.690	-1.607	-1.784	-1.756	-1.756
NH_3/N (% of DM)							138.506	130.351	145.714	144.631	144.819
R^2	0.403	0.503	0.529	0.536	0.538	0.542	0.545	0.549	0.552	0.553	0.553
$S_{y \cdot x}$	5.28	4.87	4.79	4.81	4.86	4.89	4.93	4.98	5.02	5.08	5.15

[a]Regression coefficient is significant ($P < .05$)

[1]For explanation of abreviations see Table 4.

Table 18. Multiple regression of IVDOM on Laboratory measurements for 19 corn silages in clostridial group.[1]

Parameter	Number of variables in model										
	1	2	3	4	5	6	7	8	9	10	11
	regression coefficient										
Intercept Y	71.801	64.160	72.163	73.332	43.080	-53.333	22.363	5.073	-15.912	-29.281	-32.631
ADF (% of DM)			-0.334	-0.387				0.198	0.195	0.213	0.225
ADF-N (% of N)		0.695[a]	0.779[a]	1.379[a]	1.624[a]	6.610	2.086[a]	2.263[a]	3.493	4.333	4.189
ADF-N (% of DM)				-38.351	-66.377	-405.704	-76.306	-85.430	-174.983	-237.006	-222.457
CP (% of DM)						9.617			2.561	3.963	4.189
DM (%)					0.315	0.444	0.355	0.491	0.513	0.501	0.482
Lactate (% of DM)							1.682	2.091	1.788	1.862	1.903
NDF (% of DM)						0.235	0.275	0.352	0.344	0.366	0.370
pH					0.984		1.050	1.157	1.008	1.028	1.270
K (% of DM)	-9.142[a]	-10.854[a]	-9.984[a]	-10.411[a]	-7.199	-10.260[a]	-9.591[a]	-9.396	-9.518	-10.081	-10.806
NH_3/N (% of N)										0.069	0.463
NH_3/N (% of DM)											-26.532
R^2	0.340	0.488	0.544	0.586	0.614	0.643	0.666	0.670	0.672	0.674	0.675
$S_{y \cdot x}$	4.39	3.99	3.89	3.83	3.84	3.85	3.89	4.05	4.26	4.50	4.80

[a]Regression coefficient is significant ($P < .05$).

[1]For explanation of abreviations see Table 4.

Table 19. Multiple regression of IVDOM on laboratory measurements for 55 corn silages in oxidized group.[1]

Parameter	Number of variables in model										
	1	2	3	4	5	6	7	8	9	10	11
	regression coefficients										
Intercept Y	86.148	64.330	60.325	58.610	59.447	58.097	61.027	60.701	51.284	48.413	47.869
ADF (% of DM)	-0.889[a]	-0.678[a]	-0.454[a]	-0.445[a]	-0.442[a]	-0.438[a]	-0.395	-0.398	-0.360	-0.370	-0.362
ADF-N (% of N)									0.254	0.404	0.409
ADF-N (% of DM)			-17.752[a]	-16.876[a]	-16.149	-14.084	-15.434	-19.803	-34.897	-51.884	-52.499
CP (% of DM)									0.964	1.010	1.004
DM (%)		0.333[a]	0.351[a]	0.361[a]	0.355[a]	0.363[a]	0.375[a]	0.384[a]	0.386[a]	0.423[a]	0.418[a]
Lactate (% of DM)				1.418	1.688	1.756	1.792	1.817	1.805	1.911	1.994
NDF (% of DM)							-0.073	-0.076	-0.069	-0.078	-0.084
pH											0.184
K (% of DM)								1.178		2.346	2.325
NH_3/N (% of N)						0.235	0.269	0.249	0.353	0.298	0.277
NH_3/N (% of DM)					-9.669	-25.244	-27.983	-29.905	-34.967	-32.952	-30.538
R^2	0.314	0.484	0.527	0.545	0.550	0.556	0.562	0.565	0.570	0.577	0.578
$S_{y \cdot x}$	6.04	5.28	5.11	5.06	5.08	5.10	5.12	5.16	5.18	5.20	5.26

[a] Regression coefficient is significant ($P < .05$).

[1] For explanation of abreviations see Table 4.

The R^2 of .806 for the full model was about 13% higher than was obtained for any of the other groups and the $S_{y.x}$ of 1.43 was substantially lower. The better prediction for the standard group may be due to the greater uniformity of samples and lack of abnormal fermentations within the group.

NEAR-INFRARED REFLECTANCE

Pulse points and delta OD's for areas of minimum, maximum, and least change in reflectance for six corn silages are listed in Apppendix Table 1. From these areas of change in optical density, pulse points were selected for a 10 sample calibration for IVDOM. Table 20 shows the pulse points that were examined for an optimum correlation search with respective correlations and $S_{y.x}$.

The highest correlation (.951) and lowest $S_{y.x}$ (4.04) was obtained with six pairs of pulse points labeled group A in Table 20. However, the instrument used only two pairs of pulse points for the regression. The prediction using pulse points 265 to 285 and 590 to 600 (group H, Table 20) was comparable to all six pairs (r=.943, $S_{y.x}$ =4.362). Conversion of pulse points to spectral data indicates that pulse points 265 to 285 and 590 to 600 correspond to wavelengths 2.0472 to 2.0713 and 2.2360 to 2.2397 microns respectively. The first pair (265 to 285) would reside at filter position

Table 20. Optimum correlation search employing mini calibration set of ten corn silages.

	Pulse Points	b	r	$S_{y \cdot x}$	Pulse Points	b	r	$S_{y \cdot x}$
Group	A				B			
	410-420		.951	4.035	410-420	9970.7	.814	7.125
	265-285	-2356.8			330-336			
	330-336				720-725			
	590-600	9062.4			265-280			
	720-725				139-149			
	725-729				85-89			
Group	C				D			
	2-15		.845	7.016	139-149		.916	5.244
	85-89				265-280			
	139-149				410-420			
	208-217				590-600	6773.1		
	265-285	-1818.6			720-725	66.5		
	330-336	3698.6			725-729			
Group	E				F			
	410-420	7258.0	.924	4.996	2-15		.851	6.874
	470-473				85-89			
	523-533				139-149	6000.0		
	590-600	7690.1			208-217			
	650-660				315-330			
	688-696				265-280	-3388.6		
Group	G				H			
	410-420	9999.5	.814	7.616	265-285	-2679.2	.943	4.362
	470-473				590-600	6000.0		
	523-533							
	688-696							
	725-729							
Group	I				J			
	720-729	2034.1	.813	7.139	720-725	-1055.8	.815	7.102
	410-420				725-729			
Group	K				L			
	720-729	1267.2	.789	7.385	720-729	707.5	.781	7.649
	265-280							
Group	M							
	410-420	6000.0	.811	7.168				

3 of the instrument and are in the area of the NIR spectrum where protein and starch absorb. The second pair of points (590 to 600) reside at filter position 5 and are in the area of fiber and sugar absorption.

A calibration set of 26 corn silages was selected to test the pulse points obtained from the optimum correlation search. When the full calibration set was tested employing all six pulse point pairs (group A, Table 21) a correlation of .876 and $S_{y.x}$ of 5.382 was obtained Table 21 shows that the instrument used a third pair of pulse points (410 to 420) for the prediction. This additional pair resides at filter position 4 of the instrument in an area of the spectrum where protein absorbs and corresponds to wavelengths 2.1452 to 2.1566 microns. The set of three pulse point pairs (group D, Table 21) resulted in an almost identical correlation (.879 and $S_{y.x}$ (5.38) as the regression employing six pairs. Therefore, the pulse point pairs represented in group D were selected for the prediction of IVDOM for 90 unknown corn silages. The IVDOM values determined in the laboratory versus predicted by NIR (NIRDOM) are shown in Appendix Table 2.

IVDOM was regressed on the NIRDOM with an R^2 of .367 and $S_{y.x}$ of 4.04. This compares with the prediction by ADF with R^2 of .352 and $S_{y.x}$ of 5.29 (Table 10).

Table 21. Pulse points employed for 26 sample calibration set.

Pulse points	b	r	$S_{y.x}$	Group
410-420	-6536.4	.876	5.382	A
265-285	-4690.5			
330-336				
590-600	8382.2			
720-725				
725-729				
590-600	5598.6	.380	9.864	B
265-285	-2999.6	.764	6.884	C
410-420	-6677.2	.879	5.308	D[a]
265-285	-4722.1			
590-600	8168.5			

[a]Group D is pulse point set used for final prediction of 90 corn silages.

The regression equation for TDN employed in the Virginia Tech Forage Testing Program was used to predict TDN values for the data set of 90 corn silages. The Forage Testing equation is: TDN=80.4 - 0.481 x ADF. IVDOM was regressed on TDN and an R^2 of .279 and $S_{y.x}$ of 4.31 was obtained.

Chapter IV

GENERAL DISCUSSION AND CONCLUSIONS

The screening process employed to select silage samples resulted in a wide range of silage qualities and fermentations as evidenced by the chemical composition data. The range of IVDOM from 38.5 to 74.7 is further evidence of substantial differences in silage quality and that the modified Tilley and Terry procedure was sensitive to silage differences. Such variation in corn silage quality has not been reported elsewhere in the literature. Although the data set was probably biased by the screening procedure, these data clearly indicate the range of fermentations that can occur in corn silage.

Under practical forage testing conditions, assays for predicting silage quality would be confined to relatively stable chemical constituents. Readily degradable or volatile compounds would be lost or vary with exposure to the atmosphere rather than relate to fermentation in the silo. In this study ADF and DM were the most consistent estimators of corn silage IVDOM and adding more variables did not improve the prediction enough to warrant their inclusion. Although the prediction by ADF was not extremely accurate, ADF was the best predictor found.

Regression analyses within fermentation groups indicated that other variables besides ADF and DM might be effective predictors for abnormal fermentations. The problem with grouping silages by fermentation type is that there is no accurate method to do so. Infrared reflectance offers potential in this area if the instrument can be calibrated to screen silages by fermentation type. High and low quality corn silages were found in all fermentation groups but mean IVDOM was higher for the standard group.

Although CP is commonly included in many prediction equations for feeding value, these results indicate CP adds very little to the prediction of IVDOM for corn silage. This is consistent with the findings of Marten et al. (48). Apparently CP is an important nutrient when considering balanced rations but is of little value for predicting corn silage digestibility.

Probably the biggest limitation on predicting silage quality is the lack of understanding as to the chemical constituents that consistently affect digestibility. It is known that increased grain content of corn silage will compensate for decreased digestibility of the plant portion. A reliable assay to estimate grain content may be worth investigating.

Differences in chemical composition of silages was indicated by visual observation. Black and brown silages were higher in ADF than the green-yellow silages. The pleasant smelling silages were significantly lower in ADF. The sour and musty smelling silages showed higher pH than the other aromas. Moldy silages were significantly higher in DM and pH than those without mold. However, these differences were not reflected in changes in digestibility.

In this study the NIR prediction of IVDOM was comparable to prediction by ADF or TDN. These data suggest that NIR has potential merit for rapid determinations of corn silage digestibility. The rapidity of determinations makes the NIR prediction seem attractive, however, further analyses of pulse point data is warranted. A severe limitation on the NIR technique for making predictions, is the consistancy and repeatability of laboratory measurements for an accurate calibration of the instrument. Furthermore, the regression equations developed should be tested on a randomly selected set of corn silages.

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APPENDIX

Appendix Table 1. Delta OD's and pulse points for six corn silages as determined by Versidump 2.

#20		#121		#05		#106		#174		#10	
PP[a]	OD[a]	PP	OD	PP	OD	PP	OD	PP	OD	PP	OD
2	.00468	7	.00563	2	.00390	8	.00642	4	.00500	11	.00537
44	.00021	46	.00057	82	.00000	81	.00040	83	.00000	85	.00163
89	-.00060	75	-.00122	89	-.00389	89	-.00680	89	-.00313	89	-.00175
139	.00499	129	.00724	129	.00585	129	.00799	129	.00567	129	.00584
209	.00143	171	.00208	208	.00188	190	.00123	208	.00187	190	.00062
217	-.05919	217	-.05604	217	-.06195	217	-.05838	217	-.05742	217	-.04715
337	.00594	336	.00727	336	.00651	336	.00797	336	.00708	337	.00542
312	-.00022	307	-.00019	311	-.00002	308	.00026	311	.00003	312	.00008
257	-.04029	257	.03886	257	-.04259	257	-.03989	257	-.04316	257	-.03608
417	.02111	415	.02163	416	.02184	415	.02397	416	.02348	418	.01795
466	-.00229	466	-.00266	466	.00293	466	-.00245	466	-.00252	465	.00170
473	-.04184	473	-.04433	473	-.04353	473	-.04750	473	-.04493	473	-.03608
523	.02697	523	.02790	519	.02778	523	.03053	521	.02921	528	.02164
585	.00018	588	.00005	582	-.00015	590	.00001	585	.00004	589	.00026
601	-.04972	601	-.04976	601	.05134	601	-.05063	601	-.05086	601	-.04254
708	.00618	708	.00559	711	.00596	703	.00697	708	.00669	708	.00572
674	.00012	677	.00006	678		674	-.00007	676	-.00014	677	-.00007
641	-.01527	641	-.01706	641	-.01470	641	-.01668	641	-.01584	641	-.01547
720	.00567	720	.00492	720	.00554	720	.00589	720	.00580	720	.00492
722	-.80842	722	-.80774	722	-.80616	722	-.08070	722	-.80627	722	-.80922
725	-.02046	725	-.01762	725	-.01460	725	-.01572	725	-.01526	725	-.02126
729	-.56638	729	-.56059	729	-.55573	729	-.55795	729	-.55704	729	-.56712

[a]PP=pulse points, OD=optical density.

Appendix Table 2. NIR prediction of IVDOM.

Sample no.	Laboratory DOM	NIR[a] DOM	Difference IVDOM-NIRDOM
	% of DM		
1	61.5	55.2	6.4
2	63.9	57.7	6.2
4	52.9	51.1	1.8
6	57.8	50.8	7.0
8	65.6	57.4	8.2
9	63.5	55.3	8.2
11	55.8	61.3	-5.5
15	62.1	57.1	5.0
22	62.6	60.5	2.1
24	72.2	59.0	13.2
29	55.1	59.9	-4.8
31	59.2	57.8	1.4
34	64.1	50.6	13.5
36	71.5	68.4	3.1
38	59.4	59.0	0.4
40	57.5	48.7	8.8
42	66.1	61.8	4.3
51	59.9	61.7	-1.8
55	51.6	56.3	-4.7
57	58.8	50.3	8.6
63	57.2	44.9	12.3
66	70.9	65.6	5.3
68	62.7	53.9	8.8
71	53.3	59.0	-5.7
73	58.7	63.9	-5.2
76	64.1	62.2	1.9
78	58.0	60.1	-2.1
79	59.7	56.6	3.1
81	62.8	65.3	-2.5
85	57.0	57.2	-0.2
88	55.8	58.2	-2.4
89	61.8	45.8	16.0
106	62.1	57.0	5.1

[a]Near-infrared reflectance prediction of IVDOM.

Appendix Table 2. Cont. NIR prediction of IVDOM.

Sample no.	Laboratory DOM	NIR[a] DOM	Difference IVDOM-NIRDOM
	% of DM		
111	54.5	61.9	-7.4
112	56.6	54.7	1.9
122	69.4	68.3	1.1
123	63.8	65.6	-1.8
124	55.5	60.6	-5.1
127	56.2	57.4	-1.2
128	61.1	60.8	0.3
129	57.8	54.1	3.7
132	58.0	53.8	4.2
134	56.7	49.5	7.2
135	57.3	56.8	0.5
136	60.5	57.3	3.2
138	51.7	37.4	14.3
140	57.8	59.3	-1.5
141	58.7	60.4	-1.7
142	55.3	52.3	3.0
143	64.5	62.2	2.3
144	65.8	61.1	4.7
145	66.4	63.7	2.7
146	57.4	58.4	-1.0
148	62.2	65.3	-3.1
149	63.0	60.9	2.1
150	61.9	61.7	0.2
151	59.4	60.9	-1.5
152	62.0	56.6	5.4
153	53.4	60.6	-7.2
154	57.8	59.5	-1.7
155	57.3	55.2	2.1
156	63.3	65.3	-2.0
157	70.5	71.4	-0.9
158	58.3	63.4	-5.1
159	53.4	47.1	6.3

[a]Near-infrared reflectance prediction of IVDOM.

Appendix Table 2. Cont. NIR prediction of IVDOM.

Sample no.	Laboratory DOM	NIR[a] DOM	Difference IVDOM-NIRDOM
	% of DM		
160	59.1	62.6	-3.5
162	49.0	47.6	1.5
163	49.1	46.3	2.8
164	59.5	56.4	3.1
168	66.5	68.2	-1.7
170	51.0	48.3	2.7
171	58.1	55.3	2.8
172	65.7	61.9	3.8
176	55.2	55.7	-0.5
178	62.8	54.1	8.8
179	55.7	49.5	6.2
180	52.1	59.4	-7.3
183	66.3	62.0	4.3
184	63.1	59.9	3.2
186	56.8	46.2	10.6
188	55.8	51.3	4.6
190	60.6	50.7	9.9
201	62.8	64.8	-2.0
202	65.0	61.7	3.3
203	62.1	62.4	-0.3
204	66.4	57.8	8.6
205	67.9	66.9	1.0
206	61.3	57.0	4.4
207	67.6	64.2	3.4
208	51.0	63.5	-2.5

[a]Near-infrared reflectance prediction of IVDOM.

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ASSAYS FOR DETERIORATION AND ESTIMATES OF FEEDING VALUE OF CORN SILAGE

by

Aaron J. Moe

(ABSTRACT)

Corn silages were selected from samples analyzed in the Virginia Tech Forage Testing Laboratory. A sample selection scheme employing observation of silage quality indicators (color, mold, aroma, fermentation type) was utilized to obtain a sample set encompassing a wide range in deterioration. In vitro digestible organic matter (IVDOM) was the criteria used to estimate feeding value. The full data set contained 142 corn silages which averaged 59.8 $\pm$ 6.6% and ranged from 38.5 to 74.7% IVDOM. Simple and multiple regression of IVDOM on chemical constituents were performed for the full data set and within fermentation groups. Coefficients of determination (R^2) revealed that acid detergent fiber (ADF) and dry matter (DM) explained the largest proportion of variation in IVDOM. The simple regression on the full data set resulted in R^2 of .352 and .208 with standard errors of the prediction $S_{y.x}$ of 5.29 and 5.86 for ADF and DM, respectively. Multiple regression analyses resulted in

minor improvements in $S_{y.x}$. These data suggest the inclusion of other variables besides ADF and DM in the prediction equation is unwarranted.

Corn silage IVDOM was predicted employing Near-infrared reflectance techniques. A calibration resulted in R^2 of .77, $S_{y.x}$ of 5.38. The calibration equation was tested for the prediction of IVDOM for an additional 90 corn silages. The regression of NIR predicted IVDOM resulted in an R^2 of .37 and $S_{y.x}$ of 4.04. The prediction of corn silage IVDOM by NIR or ADF were similar.