Fermentation Analysis: Use and Interpretation

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Abstract

The fermentation qualities of silages have been described by research laboratories for many years. This information has generally not been available for routine field use in the United States by nutritionists. Cumberland Valley Analytical Services, Inc. recently made available an analysis package that describes the fermentation characteristics of silages. Fermentation characteristics reported include dry matter **(DM)**, pH, titratable acidity, ammonia, lactic acid, acetic acid, propionic acid, butyric acid and iso-butyric acid. This information may be valuable in certain situations as a comparative "report card" on silage management practices and as a troubleshooting tool. It provides limited information for ration balancing purposes. Data on over 3600 analyses are summarized by discrete DM ranges. Fermentation characteristics of silages are very significantly related to moisture level. The pH is shown to not be a sensitive index of fermentation quality. The relationship of high legume silage moisture to the presence of butyric acid and elevated ammonia levels is demonstrated. Soluble protein is shown to be a poor indicator of fermentation quality and is not related to ammonia content of haycrop silages.

Introduction

Characterization of feedstuff quality is of chief importance in allowing the nutritional advisor to assess the performance of farm management in harvesting and storing of forage crops, as well as in understanding how feeds will function in a ration. Traditional forage evaluation centers on nutrients such as moisture, fiber, and protein. These indexes of forage quality do not allow us to adequately describe forage fermentation and its potential impact on animal performance. In recent years, more attention has been paid to the pH of a fermented feed as an indication of the quality of the fermentation. Few other evaluative tools have been available to the field person in attempting to describe forage fermentation.

Researchers evaluate forage fermentation by looking at not only pH, but the type and quantity of fermentation acids produced. Protein breakdown during fermentation is evaluated by ammonia, soluble protein, and true protein assays. Until recently these tools for evaluating forage fermentation had been limited to the research lab due to the cost and availability of commercial labs to run these assays.

Cumberland Valley Analytical Services **(CVAS)**, Inc., of Hagerstown, Maryland introduced an analytical package several years ago with the goal of making available a set of tests at a reasonable cost that would allow for evaluation of forage fermentation. This "fermentation analysis" package (Figure 1) includes DM, pH, ammonia, titratable acidity, lactic acid, acetic acid, propionic acid, butyric acid, and iso-butyric acid. Additionally, ethanol may be requested in this analysis package as an indicator of yeast activity. Approximately 5000 samples have been

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run for this fermentation analysis package since its introduction. Data from approximately 3600 more recent analysis are summarized for the purposes of this review. Conclusions are specific to this data set and may not be representative of the population of forages as a whole. Samples are not submitted randomly – often the suspicion of a problem or a poor fermentation generates a request for a fermentation analysis.

Methods

The objective of the fermentation analysis is to offer information at a low cost with quick turnaround that justifies general field use. It was our intent to allow people working at the field level to be able to characterize the nature of the fermentation that a silage went through and to make general observations and recommendations based on that information. In order to keep the fermentation analysis package price low and be able to process a large number of samples, methods were chosen based on simplicity and cost. Analytically, the method of choice for determination of fermentation acids is high pressure liquid chromatography. This method is not practical in forage analysis due to cost and difficulty of high throughput of samples. Gas chromatography **(GC)** is also a preferred method, but running lactic acid can be problematic. We have chosen to use GC for determination of acetic acid, propionic acid, butyric acid, and iso-butyric acid. We currently use a Shimadzu gas chromatograph with two flame ionization detectors. Packed columns provided by Supelco are used.

Lactic acid is determined using a lactate-oxidase peroxide method on a YSI 5700 select biochemistry analyzer. This unit is specific for the L(+) isomer of lactic acid. Bacteria will produce either D(-), L(+), or a mixture of the two isomers of lactic acid. Which isomers are produced depends on the type of bacteria and pH. *Lactobacillus plantarum*, one of the most commonly isolated species of bacteria in silage, produces a slight excess of the D(-) isomer (McDonald et al., 1991). Some bacteria, such as *Enterococcus faecium*, produce only the L(+) isomer (William Mahanna, Pioneer Hi-Bred International, Inc., Johnston, IA; personal communication). Our method assumes an approximately equal mixture of the two isomers; we evaluate for the L(+) and multiply the result by 2 for total lactic acid. In certain situations where predominating strains of bacteria produce a preponderance of one isomer, our methods may overestimate or underestimate the amount of lactic acid present.

Twenty-five grams of wet silage material are blended in 200 grams of distilled water for approximately two minutes. Material is filtered through a coarse, fast-flow filter paper and the filtrate is collected. This material is then partitioned to four different processes (Figure 2). A sample of the filtrate is run through a micro-filter and then 0.1µl of sample is injected into the GC for determination of volatile fatty acids. A 0.25 µl sample is introduced to the biochemistry analyzer for determination of lactic acid. Ammonia is determined by running a 50 ml sample through the Foss Kjeltec 1030 distiller / titrator. pH and titratable acidity are determined concurrently on a 30 ml sample using a Mettler DL20 titrator.

Sample Handling for Fermentation Analysis

Sampling requirements for fermentation analysis would be the same as for other nutrient analyses. The preferred forage sample size is 250 grams or more. This allows for sufficient material for duplicate extractions with enough for DM determination as well as material for standard nutrient analyses. Optimally, a sample is placed into a plastic bag and air is squeezed out as much as possible prior to sealing the bag. The sample would be frozen and then sent overnight with an ice pack. In many farm evaluation situations the freezing and overnight mailing of samples may not be practical. Samples pulled from inside a stable silage mass may be sealed in a plastic bag with air removed and sent to the lab within two days with minimal change in the fermentation analysis. We have evaluated sample stability by placing a sample in a sealed bag with air removed and storing it at room temperature in the lab. Sub-samples were removed and tested over a period of days with little variation in results. Samples that are drier or are potentially aerobically unstable will require better handling and quicker transit time to the lab in order to minimize changes. An aerobically unstable sample will experience dramatic changes in several days without proper handling.

Use of the Fermentation Report

There are those that argue that while the fermentation report is interesting, it is of little value, providing no information that can be used directly in the ration balancing process. While it is generally true that the fermentation data have little direct application, this challenge avoids the true value of the report. The fermentation report is meant to provide a comparative evaluation that allows the user to better characterize the silage and to lend insight into possible DM intake and performance problems. A silage at 30% DM that has 1.5 % butyric acid and 18% ammonia nitrogen as a percentage of total nitrogen will be utilized differently than a silage at the same DM level that has no butyric acid and 9% ammonia nitrogen. The degree or extent of an adverse fermentation can be better determined by the fermentation analysis than by visual and olfactory observation alone.

A second, and perhaps more important application of the fermentation report is as a "report card" on the management of the silage making process. The fermentation end-products are a summary of all conditions that effected the silage making process, including plant maturity, moisture, epiphytic bacteria activity, additive use, ambient temperature, packing, and face management. Significant breakdowns in the management of the silage making process will show up as silage with less desirable fermentation characteristics. The farm adviser can use the information gained from the fermentation analysis to document on a third party basis the quality of the silage and to challenge a farmer to better silage making practices. Quality forage is the basis of profitable animal production.

The type and degree of fermentation will significantly effect the amount of DM recovery from the silage making process. Herbage that is ensiled properly exhibits a fermentation where pH drops rapidly and homofermentive bacteria predominate. Lactic acid should be a significant end-product of these fermentations. Silages that have high levels of acetic, propionic, butyric, or iso-butyric acids imply conditions where DM recovery from the silage making process may be poor.

Nutritional advisors with an Eastern U.S. feed concern recently adopted the fermentation analysis as a tool in advising farmers on silage making issues. The fermentation analysis revealed that moisture levels at ensiling were too high. Adverse fermentations were resulting. The fermentation analysis was used to document these problems and to be an evaluation tool as management practices were changed. In the following crop year, there was a concerted effort by producers to put up silage material at more appropriate moisture levels. With only small alterations in the moisture level at ensiling, quality of fermentation improved and increased animal productivity followed. Not only did the fermentation analysis point out the need for proper moisture at ensiling, but it challenged the nutritional advisor and producer both to focus on good silage management practices.

Nutritional Considerations

Fermentation Acids

Dry matter intake may be limited due to fermentation characteristics. It is well known that clostridial fermentations resulting in the creation of butyric acid and elevated levels of ammonia are characteristic of silages with poor animal acceptance. It is suspected that the protein breakdown products, such as ammonia, amines, and amides, may be respons-ible for limiting intake. Butyric acid itself may not significantly limit intake but may be a marker for protein degradation products.

Less certain is the effect of high levels of lactic or acetic acids on animal intake. Research has proven that the addition of acids, such as lactic, acetic, and propionic acid to silages prior to feeding will reduce intake (Table 1). Intake of whole frozen (then thawed) corn plants is significantly higher than the same material fed as silage. The addition of acids to either fresh or frozen whole plant corn silage significantly reduced intakes (Erdman, 1993). Richard Erdman of the University of Maryland in a review of silage pH and intake studies developed a regression for adjusting DM intakes based on the pH of a silage: DM intake a(% of bodyweight) = $-0.18 + 0.88\text{pH} - 0.077\text{pH}^2$. The degree to which intake is limited by particularly high levels of acids is, however, open to question.

Personal communications from individuals in the field suggest that there may be significant intake problems associated with some silages that have acetic acid levels above 5%. The mechanism is not understood. The acetic acid may not itself be a problem but may be a marker. It is recognized that those fermentations that produce excessive levels of acetic acid are more prolonged and are less conserving of silage DM. There are differences in the utilization of fermentation acids by the rumen. Acetic acid is not fermented in the rumen, whereas one form of lactic acid is fermented by rumen bacteria under normal conditions (Muck, 1993). Lactic acid may be a problem in silages where it exceeds 10% of DM, although that occurs only in rare fermentations in North America. Many feeding situations utilize silages with high acid content with no apparent problems. Feed bunk management, ration parameters, and associative affects of feedstuffs may determine whether high silage acid levels may be a problem in any given feeding situation.

It must be noted that silages that are higher in lactic acid with minimal acetic and propionic acid, what we consider "better" fermentations, may actually be more aerobically unstable. Lactic acid is not a good antimycotic. A certain amount of acetic acid is desirable in order to minimize possible growth of yeast and mold organisms. Poor fermentations with elevated butyric acid levels are actually much more aerobically stable.

Yeast end-products, such as methyl- and ethyl- acetates which resemble the smell of fingernail polish remover, are compounds that may also be present and limit DM intake (Seglar). Ethanol is a primary yeast end-product that may be intake limiting. Yeasts are responsible for much of the secondary heating of silages exposed to air and associated DM losses.

Ammonia

While there is no current effort to look at ammonia or nonprotein nitrogen **(NPN)** as independent variables in most ration balancing programs, there may be justification to give more consideration to evaluating ammonia in forages. Ammonia would be part of the "A" protein fraction along with amino acids and peptides. These components are buffer soluble, as well as true protein such as albumins and globulins (Asplund, 1994). Ammonia is utilized differently than peptides and true proteins. It has value as a nitrogen source for bacterial growth, but beyond what is utilized by rumen bacteria, there is an energy and metabolic cost to the animal.

Traditionally nutritionists have looked at soluble protein as the most cost effective means of estimating a functional pool of ruminally degraded protein. Soluble protein has also been used to evaluate retention of protein quality in fermented silage.

Forage evaluation data compiled by CVAS would indicate that there is significant variation in the quality of protein in the soluble fraction. In Figure 4, one can observe a very strong relationship between moisture level of legume forage and the ammonia nitrogen as a percentage of total nitrogen. This would be expected as there are more clostridial and proteolytic organisms active at higher moisture levels. However, there is little correlation between soluble protein and moisture level, indicating that the soluble protein test is not sensitive to the quality of the protein in the soluble fraction (Figure 6). It would not be a good predictor of ammonia or proteolytic activity during the forage wilting and fermentation process. In Figure 5, individual ammonia and soluble protein data are plotted for over 1000 legume samples. No particular trend is apparent. The r^2 on the correlation between soluble protein and ammonia is less than 0.01% for the data. It appears from the data that there is little change in the percentage of protein that is soluble across DM levels and that the percentage of ammonia nitrogen in the soluble pool increases at higher moisture levels. It might be inferred that the proteolytic activity of bacteria is primarily on the soluble fraction of protein.

Ammonia levels do not vary significantly in corn silage (Figure 3). Due to the high concentration of water soluble carbohydrates in corn silage, pH tends to drop fast during fermentation and to a level that inhibits activity of most clostridial organisms. It is unusual to see more than trace levels of butyric or iso-butyric acid in corn silage (Table 2).

Estimating Non-fiber Carbohydrate (NFC) Components

There has been some use of the total volatile fatty acid level to infer the make-up of the NFC fraction of a fermented feed. Non-fiber carbohydrate includes primarily the starch, sugar, soluble fiber, and organic acid content of the plant. It is not valid to test for starch, sugar, and fermentation acids, and then by difference, arrive at a soluble fiber value. First, the fermentation acids are determined on a wet forage sample. A traditional forage analysis determines nutrients on material that has been dried. The drying process will drive off 60% to 90% of the acetic acid. Eighty to almost 100% of the propionic, butyric, and iso-butyric acids are driven off by drying. The NFC of dried material will not be the same as wet. Secondly, the fermentation acids that are typically determined do not include the plant organic acids which are variable and may account for up to 10% of DM. Procedures are under development that will also allow commercial forage laboratories to report a soluble fiber value.

Use of the Fermentation Analysis as a Research Tool

The testing of fermentation acid levels, pH, and ammonia have been used by researchers for many years to describe the nature of fermentations. These data are the basis for deter-mining the significance of management practices and forage treatments in effecting the probability of improved fermentation. Too often, however, nutritionists and field advisors attempt to make comparisons or determine differences in treatments based on uncontrolled experiments run with few or no replicates. Conclusions based on this approach are questionable and are potentially incorrect.

Review of Fermentation Analysis Data

Significance of Moisture to Fermentation Outcome

The significance of level of moisture in providing conditions opportune to various epiphytic organisms can not be overstated. Fermentation end-products are significantly related to moisture level because of the epiphytes supported at those moisture levels. Tables 2 and 3 list fermentation analyses for corn silage, legume silage, grass silage, and high moisture corn by DM ranges. Most evaluations vary significantly by DM of the plant material, with the exception of pH and ammonia in corn silage. In evaluating any given fermentation analysis, it is important to compare it to sample averages for similar DM levels. What would be an expected fermentation outcome at 38% DM in a legume silage would not be the same if the material were ensiled at 30% DM. It is important to note that forage fermentation is a dynamic process and the outcome is influenced by the interaction of many different factors. Fermentations may vary considerably from "average" values but still be reasonably efficient and provide for excellent stability.

The pH as an Index of Fermentation Quality

The pH has traditionally been used to evaluate the quality of a fermentation. It is a fast and inexpensive test to run and can easily be run at the farm. While pH in a broad sense can aid in differentiating between a good and poor fermentation, it is limited in the infor-mation that it can provide. In Figure 7, average pH and total fermentation acids are graphed by DM range in corn silage. Average pH levels by dry matter range do not vary by more than 0.14 pH units from < 26 to 38% DM. In that same range total acids range from 10.5 to 6.4% of DM. pH is somewhat more descriptive in legume forages but only varies by 0.47 pH units from 24 to 52% DM; while in that same range total fermentation acids varied from 11 to 4.5% of DM (Figure 8). The pH, as an evaluative tool, is also limited in that it can not tell us about the rate of change to arrive at a terminal pH (Mahanna, 1993). The faster the drop in pH, the more DM is conserved in the fermentation process.

The Difference Between pH, Total Acid Level, and Titratable Acidity

The relationship between pH and the amount of acids in a feed material is not as strong as one might expect. The pH measures the hydrogen ion concentration or the ratio of hydrogen to hydroxyl ions (H+ to OH-). A forage fermentation may have a high ratio of hydrogen ions to hydroxyl ions but not have a large quantity of hydrogen ions (low pH, low acid level). In corn silage which has little buffering capacity, it does not take a lot of acid to reduce the pH to 4. Figure 7 shows the relationship of pH to total acid level in corn silage. There is little effect on pH from increasing fermentation acid levels. In legumes, the same relationship holds true (Figure 8) but not to the same degree.

Titratable acidity is an evaluation that has perhaps minimal value when pH and total acid levels are available. Titratable acidity for our use is defined as the mili-equivalents of base (0.1 M NaOH) necessary to titrate the pH of a silage sample to 6.5. It measures the total of all hydrogen ions neutralized in order to bring pH to 6.5 and would account for the strength of the acids present. Titratable acidity is correlated very closely to total acid levels in corn silage (Table 2; Figure 9) and high-moisture corn (Table 3). There is almost a one-to-one correspondence in those materials. In legumes (Table 10) this does not hold true due to proteins and other compounds that buffer the silage material.

Variation in Type and Amount of Fermentation Acids

Figure 11 shows the relationship between total acids, lactic acid, and acetic acid in corn silage by DM range. One can observe a very strong relationship between DM range and total acid content. Total acid content is greater than 10 at dry matter levels below 26% and drops to less than 5% at DM levels greater than 40%. Up to 36% DM, however, lactic acid remains relatively constant. The level of acetic acid increases steadily as DM decreases. These changes in acetic acid level are not due directly to DM differences, but the conditions that are created favor certain epiphytic organisms and their resulting end-products of fermentation. The level of propionic and butyric acids in corn silage is small in most situations (Table 2). Elevated levels would indicate a fermentation problem.

In Figure 12, it is observed again that type and amount of fermentation acids are directly related to DM content in legumes. Levels of butyric acid and acetic acid increase significantly in silages with DM levels below 32%. The data on legumes was evaluated to determine if there was a particular DM level below which there was a significantly higher probability of fermentation problems. A fermentation problem or failure was defined as one where butyric acid was observed at greater than 0.25% of DM. Table 4 shows the percentage of legume samples that had butyric acid levels less than 0.25% by DM range. There appears to be a break point at about 32% DM. Below 32% DM, there was a probability of 55% or less that a fermentation success would be observed. Above 32% DM the probability of success jumped to 74% or more.

Conditions that determine whether clostridial activity occurs include the DM of the crop, buffering capacity, and water soluble carbohydrate **(WSC)** level (Muck, 1998). In order to observe a fermentation success at lower dry matter levels it is necessary to have higher levels of WSC. The WSC of legumes evaluated for fermentation analysis is not known, but we can generalize about WSC levels by looking at relative feed value **(RFV)**. Legumes with higher RFV would probably have higher WSC levels at ensiling. In Table 4, the RFV of legumes were averaged by DM range for those samples where fermentation success was observed (< 0.25% butyric acid) and where fermentation failure (> 0.25% butyric acid) was observed. The average RFV of samples that experienced fermentation failure was on average 10 to 20 RFV units lower than where fermentation success was observed. Using ammonia level as a criteria of fermentation success or failure produced similar results. Legumes can be put up under wetter conditions successfully if the WSC level is high and other conditions necessary for good fermentation are met. It must be noted, however, that the less mature haycrop forage that may offer higher WSC also often has higher buffering capacity (Mahanna, 1993), which makes it more resistant to pH change and offers clostridia more opportunity to proliferate.

Conclusions

The following conclusions would be offered concerning the use of fermentation analysis and the interpretation of fermentation analysis data:

1) Fermentation analysis is a diagnostic tool that will allow the nutritionist to better characterize problem forages and their possible contribution to DM intake problems.

2) Fermentation analysis can be used as a management "report card" on the silage making process. It allows the advisor and producer to focus on potential weaknesses in management that may need to be corrected.

3) The fermentation analysis provides limited information that has direct application to the ration balancing process.

4) Evaluation of fermentation end-products is a common research tool, but the field person needs to be careful in using fermentation analysis to draw conclusions about treatments and practices.

5) The outcome of a forage fermentation is significantly related to DM level at ensiling due to the epiphytic organisms that are supported. Total acids, as well as types of acids present, are significantly correlated to DM level.

6) In evaluating any given fermentation analysis, it is important to compare the given sample analysis to averages for samples of similar DM levels and against ideal target levels.

7) The pH is not by itself a good evaluator of the fermentation process. Total acid level and the types of acids present are a much better means of characterizing a forage fermentation.

8) Soluble protein may not be a good predictor of fermentation extent or quality. There is no apparent correlation between ammonia and soluble protein in legumes. Ammonia level is a good predictor of proteolysis and fermentation quality.

9) Fermentation analysis data would suggest that for legumes, forage DM levels below 32% have a significantly higher risk of fermentation failure (elevated butyric acid and ammonia levels).

References

Asplund, J.M. 1994. Principles of protein nutrition of ruminants. CRC press, Boca Raton, FL.

Erdman, R. 1993. Silage fermentation characteristics affecting feed intake. In: Proc. National Silage Production Conference. Syracuse, New York. p. 210-219.

Mahanna, B. 1993. Troubleshooting silage problems. Proceedings Four State Applied Nutrition Conference, June 29-30, l993, LaCrosse, WI.

McDonald, P., A.R. Henderson, and S.J.E. Heron. 1991. The biochemistry of silage. Chalcombe Publications, Marlow Bottom, Bucks.

Muck, R. E. 1988. Factors influencing silage quality and their implications for management. J. Dairy Sci. 71:2992-3002.

Muck, R. E. 1993. Ensiling and its effect on crop quality. In: Proc. National Silage Production Conference. Syracuse, New York. p. 57-66.

Seglar, B. Coping with catastrophic ensiled forage losses: case studies. Pioneer Hi-bred International, Inc., Johnston, IA.

Table 1. Silage quality factors that have been studied that affect forage intake (Erdman, 1993).

Factor	Treatment	Effect on Intake
Water	Increased silage concentration	Decrease
	Addition to feed	None
	Feeding dried silage	No improvement
Silage extracts and effluents	Addition to hay	Decrease
Lactic acid	Addition to silage	Large decrease
	Increased silage concentration	Slight increase
Sodium lactate	Addition to silage	Silage decrease
Acetic acid	Addition to silage	Decrease
	Increased silage concentration	Decrease
Sodium acetate	Addition to silage	None
Sodium propionate	Addition to silage	None
Butyric acid	Increased silage concentration	Decrease
Ammonia	Addition to silage	Decrease
	Added at ensiling	None
Soluble amines	Increased silage concentration	Decrease
pH	Partial neutralization of silage prior to feeding	Increase
	Added acid	Decrease
	Decreased silage pH	Decrease

Table 2. Average values for fermentation analysis by moisture level within feed class.

Corn Silage

DM Range (%)	Number	NH_4 Nitrogen (% of DM)	NH_4 Nitrogen (% Total Nitrogen)	pH	Titratable Acidity (meq/g)	Lactic Acid (% of DM)	Acetic Acid (% of DM)	Propionic Acid (% of DM)	Butyric Acid (% of DM)	Total Acids (% of DM)	Lactic Acid (% Total Acids)
< 26	156	0.77	8.39	3.99	10.23	5.05	4.89	0.40	0.12	10.5	48.3
26-28	215	0.72	8.15	3.86	10.27	5.42	4.21	0.44	0.07	10.1	53.5
28-30	351	0.78	9.15	3.86	9.54	5.17	3.79	0.40	0.03	9.4	55.1
30-32	355	0.68	8.00	3.89	8.35	5.15	3.19	0.30	0.03	8.7	59.4
32-34	313	0.63	7.61	3.90	7.47	4.73	2.59	0.20	0.02	7.5	62.7
34-36	231	0.80	9.41	3.86	6.96	4.77	2.36	0.17	0.04	7.3	65.0
36-38	154	0.71	8.59	4.00	5.98	4.21	2.02	0.14	0.03	6.4	65.8
38-40	112	0.66	7.81	4.09	4.79	3.56	1.69	0.08	0.02	5.4	66.5
> 40	198	0.65	7.80	4.17	3.66	3.20	1.30	0.05	0.03	4.6	69.9

Legume Silage

DM Range (%)	Number	NH_4 Nitrogen (% of DM)	NH_4 Nitrogen (% Total Nitrogen)	pH	Titratable Acidity (meq/g)	Lactic Acid (% of DM)	Acetic Acid (% of DM)	Propionic Acid (% of DM)	Butyric Acid (% of DM)	Total Acids (% of DM)	Lactic Acid (% Total Acids)
< 24	48	5.25	27.95	5.39	3.77	3.04	4.18	0.64	2.10	10.0	30.5
24-28	116	4.57	22.97	3.86	4.45	4.26	0.61	1.64	1.61	11.0	40.7
28-32	212	3.33	16.40	4.91	4.63	4.87	3.80	0.33	0.91	9.9	49.1
32-36	191	2.41	11.99	4.84	4.38	5.26	2.96	0.15	0.15	8.7	60.4
36-40	228	1.90	9.59	4.70	3.97	4.95	2.15	0.09	0.20	7.4	67.0
40-44	172	1.63	8.03	4.76	3.44	4.83	1.62	0.06	0.09	6.6	73.2
44-48	99	1.68	7.89	4.77	3.25	4.42	1.45	0.04	0.01	5.9	74.7
48-52	75	1.35	6.59	4.90	2.47	3.39	1.04	0.03	0.05	4.5	75.2
> 52	86	1.11	5.44	5.50	2.01	2.06	0.68	0.04	0.02	2.8	73.6

Table 3. Average values for fermentation analysis by moisture level within feed class.

Grass Silage

DM Range (%)	Number	NH_4 Nitrogen (% of DM)	NH_4 Nitrogen (% Total Nitrogen)	pH	Titratable Acidity (meq/g)	Lactic Acid (% of DM)	Acetic Acid (% of DM)	Propionic Acid (% of DM)	Butyric Acid (% of DM)	Total Acids (% of DM)	Lactic Acid (% Total Acids)
<24	45	4.05	26.47	5.03	5.25	3.34	4.02	0.72	1.60	9.7	34.5
24-28	66	2.44	16.26	4.73	5.79	4.49	3.15	0.37	0.81	8.8	50.9
28-32	100	1.51	10.54	4.51	5.48	4.57	2.49	0.25	0.40	7.7	59.3
32-36	73	1.34	9.12	4.57	4.45	4.72	2.05	0.13	0.34	7.2	65.2
36-40	44	1.37	9.33	4.59	3.60	4.59	1.59	0.14	0.16	6.5	70.8
40-44	34	0.93	6.24	4.60	2.93	4.09	1.10	0.03	0.05	5.3	77.6
> 44	33	1.03	6.66	4.85	2.31	2.90	1.10	0.03	0.02	4.1	71.6

High Moisture Corn

DM Range (%)	Number	NH_4 Nitrogen (% of DM)	NH_4 Nitrogen (% Total Nitrogen)	pH	Titratable Acidity (meq/g)	Lactic Acid (% of DM)	Acetic Acid (% of DM)	Propionic Acid (% of DM)	Butyric Acid (% of DM)	Total Acids (% of DM)	Lactic Acid (% Total Acids)
<64	11	0.53	6.02	3.66	3.26	2.35	0.97	0.05	0.01	3.4	69.1
64-68	28	0.53	5.72	4.04	1.51	1.24	0.48	0.02	0.01	1.8	68.9
68-72	66	0.48	5.20	4.38	1.12	0.96	0.33	0.04	0.02	1.4	68.5
72-76	83	0.30	3.23	4.26	1.03	0.84	0.24	0.01	0.00	1.1	76.4
76-80	23	0.20	2.15	5.07	0.42	0.43	0.22	0.01	0.00	0.7	61.4

Table 4. Probability of butyric acid < 0.25% by dry matter level in legume silage.

DM range (%)	% Samples < 0.25% butyric acid	Average RFV < 0.25% butyric acid[1]	Average RFV < 0.25% butyric acid[2]
<24	39.5	96	112
24-28	44.3	125	103
28-32	54.9	125	109
32-36	74.4	130	113
36-40	82.9	133	120
40-44	90.7	131	120
44-48	98.5	135	99
48-52	97.1	132	110
> 52	98.9	127	124

[1]The average relative feed value (RFV) of samples with butyric acid levels at less than 0.25% on a DM basis (fermentation success).

[2]The average relative feed value (RFV) of samples with butyric acid levels at greater than 0.25% on a DM basis (fermentation failure).

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Maugansville, MD 21767 301-790-1980 **Sample No: 0169116**

QUALITATIVE FORAGE ANALYSIS

Legume	Value	Unit	Average for DM Range 32 - 36%
Dry Matter	33.7	% DM	
pH	5.34		4.84
Titratable Acidity	3.07	meq/g	4.38
Lactic Acid	1.90	% of DM	5.26
Acetic Acid	4.00	% of DM	2.96
Propionic Acid	0.57	% of DM	0.15
Iso-butyric Acid	0.12	% of DM	0.06
Butyric Acid	3.42	% of DM	0.34
Total Acids	10.01	% of DM	8.70
Lactic Acid, % of total acids	19.0		60.4
Ammonia N, Crude Protein Equivalent	4.8	% of DM	2.41
Ammonia N, % of total N	24.4		12.0

George Johnson
Empire Dairy Nutrition
1113 Lake Road
Dryden, NY 13053

Sample : Haylage - Middle Trench
Farm Name : Peta Holsteins
Smpld/Rcvd : 03-08-2000 / 03-09-2000
Complete : 03-10-2000

Figure 1. Example of fermentation analysis report.

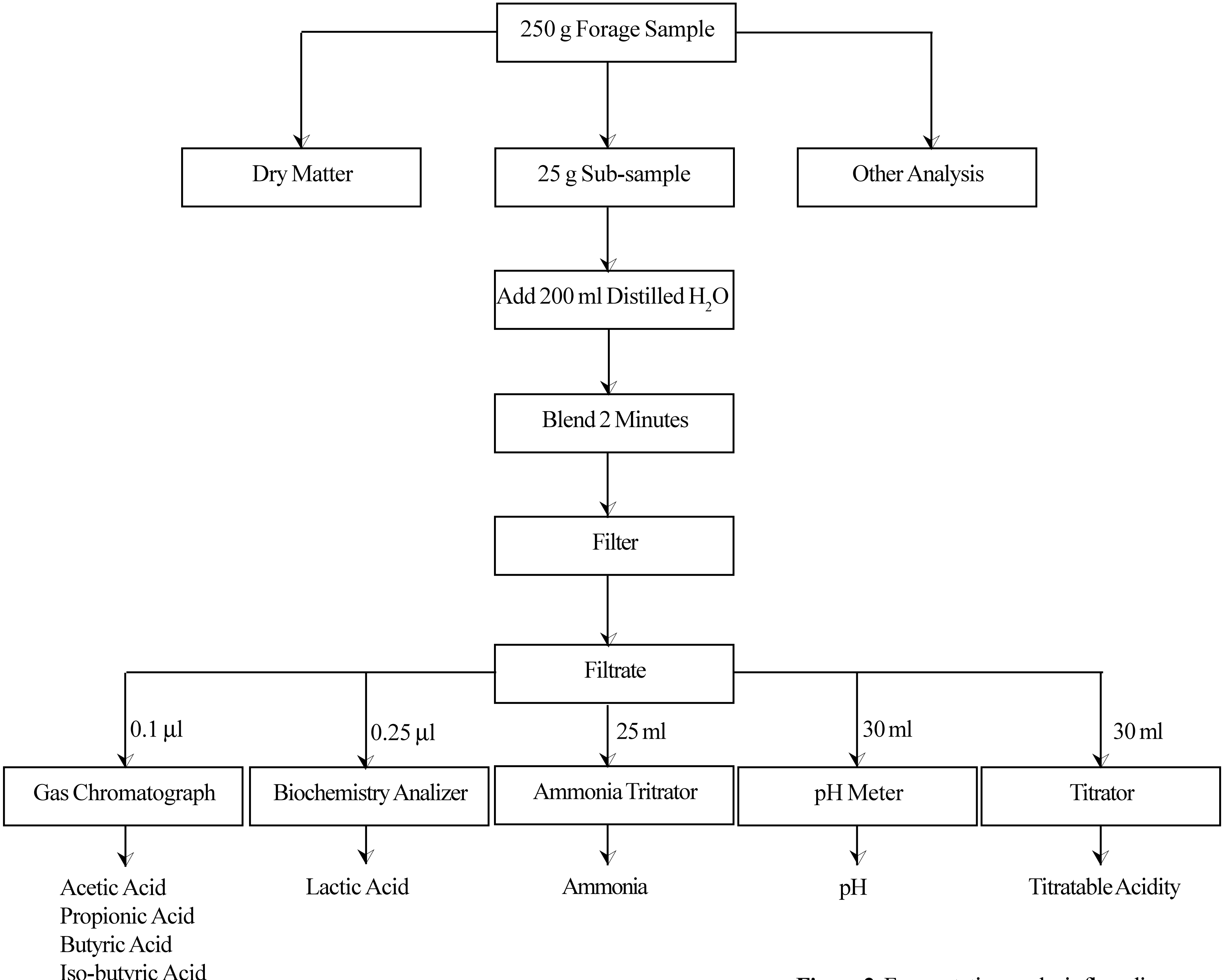

Figure 2. Fermentation analysis flow diagram

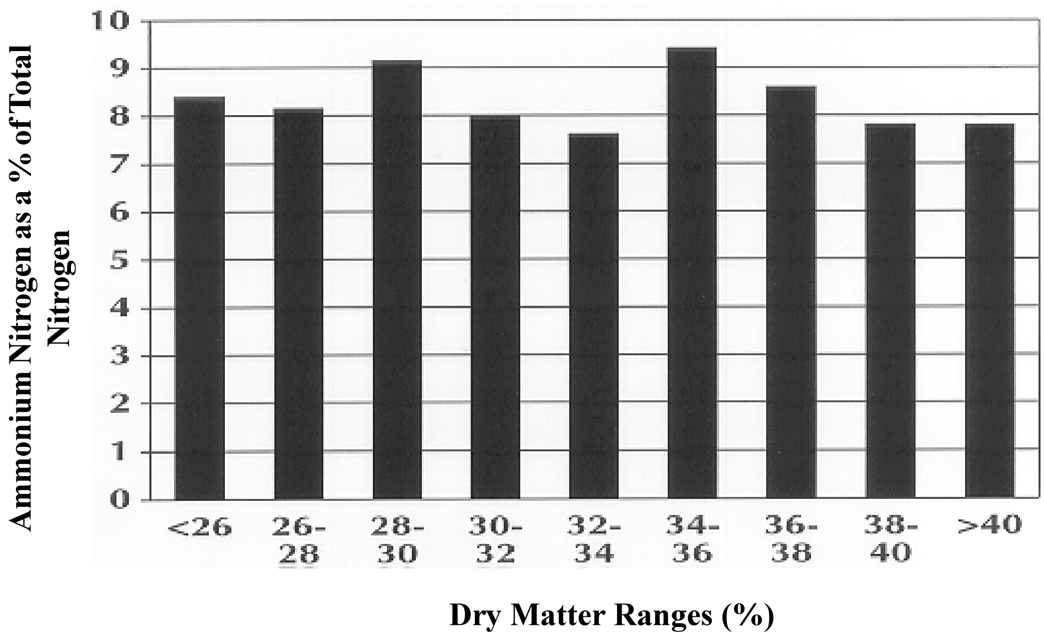

Figure 3. Ammonia nitrogen as a percentage of total nitrogen by dry matter level in corn silage.

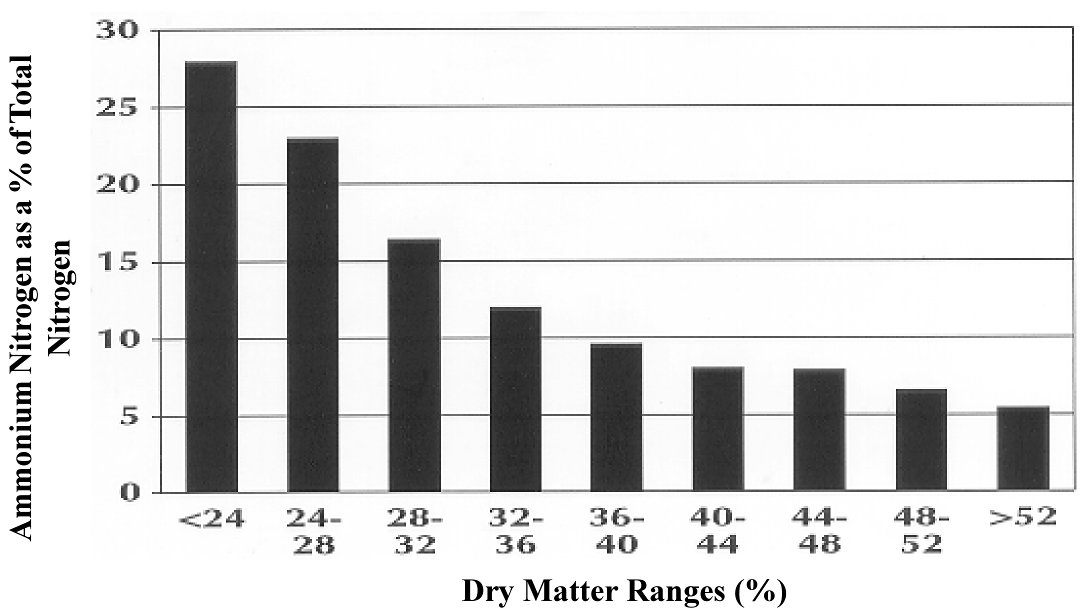

Figure 4. Ammonia nitrogen as a percentage of total nitrogen by dry matter level in legume silage.

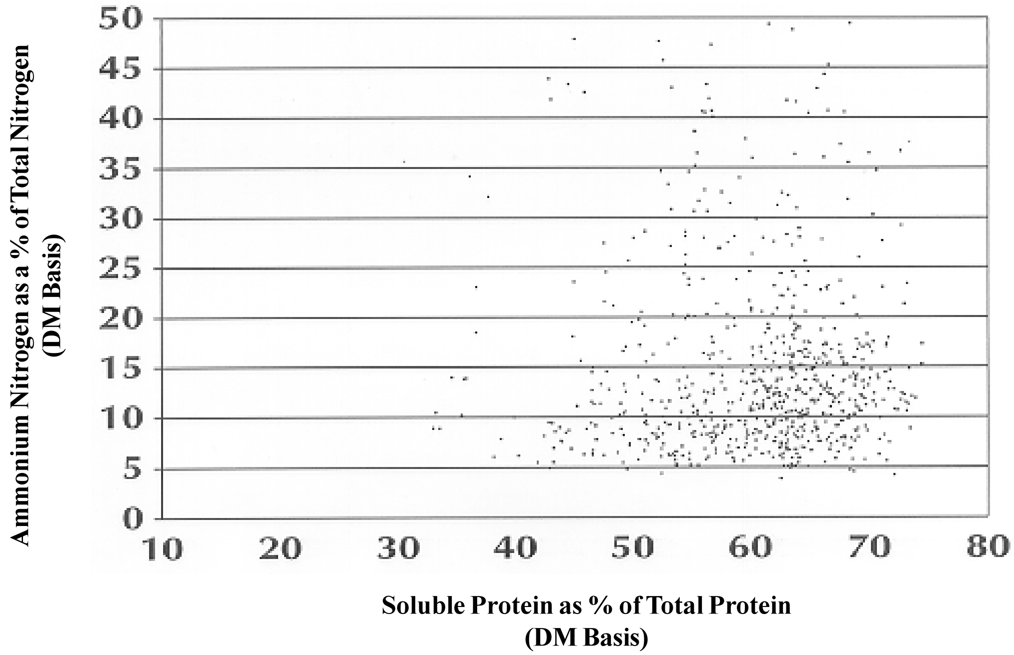

Figure 5. Ammonia nitrogen compared to soluble protein in legume silage samples < 40% dry matter.

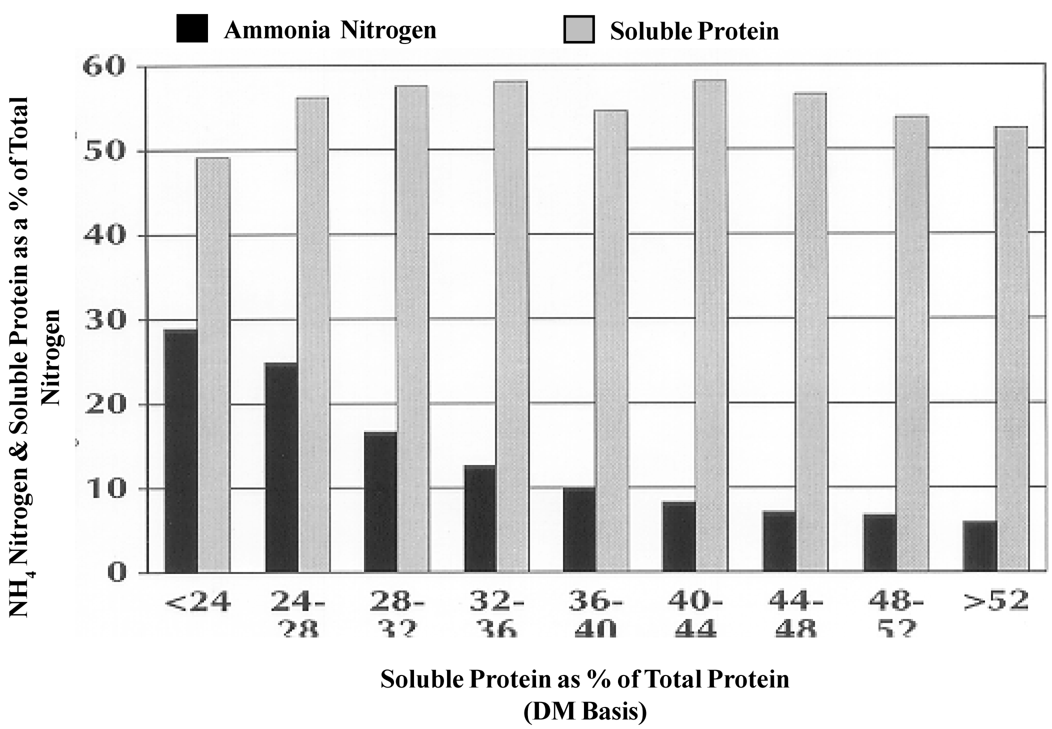

Figure 6. Ammonia nitrogen and soluble protein by dry matter level in legume silage.

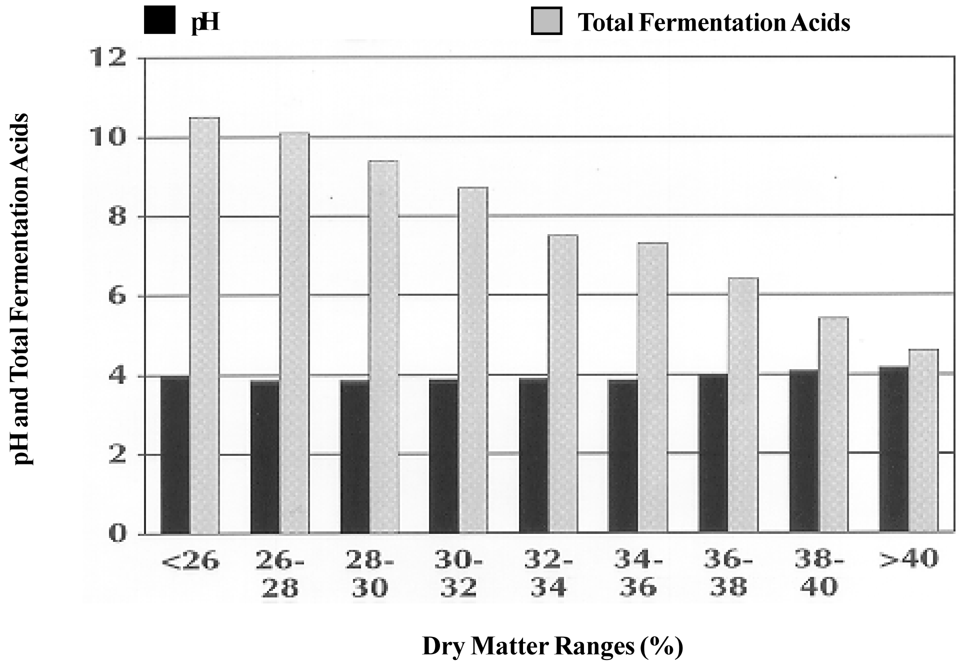

Figure 7. The pH and total fermentation acids (% of DM) by dry matter range in corn silage.

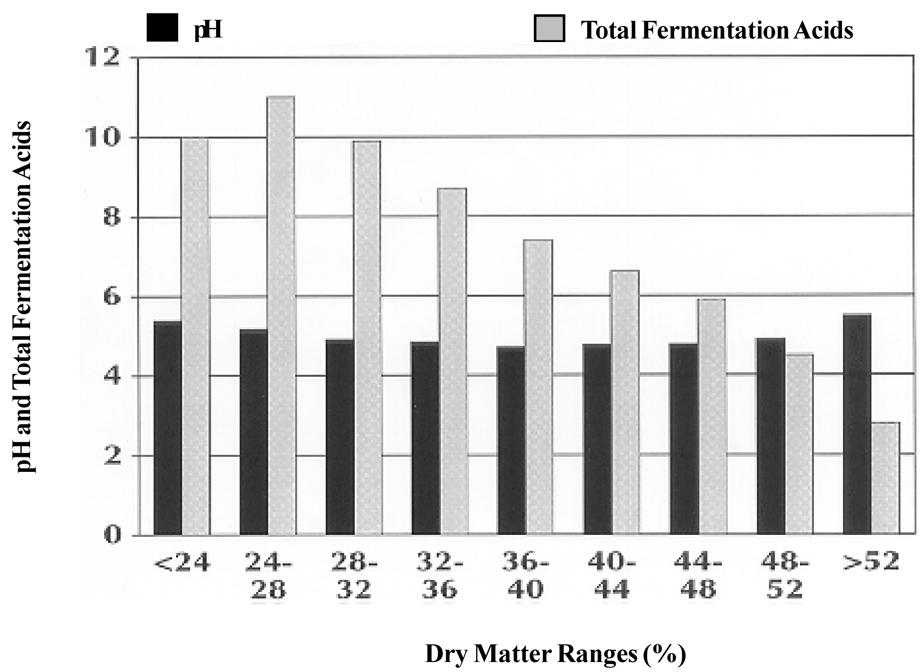

Figure 8. The pH and total fermentation acids (% of DM) by dry matter range in legume silage.

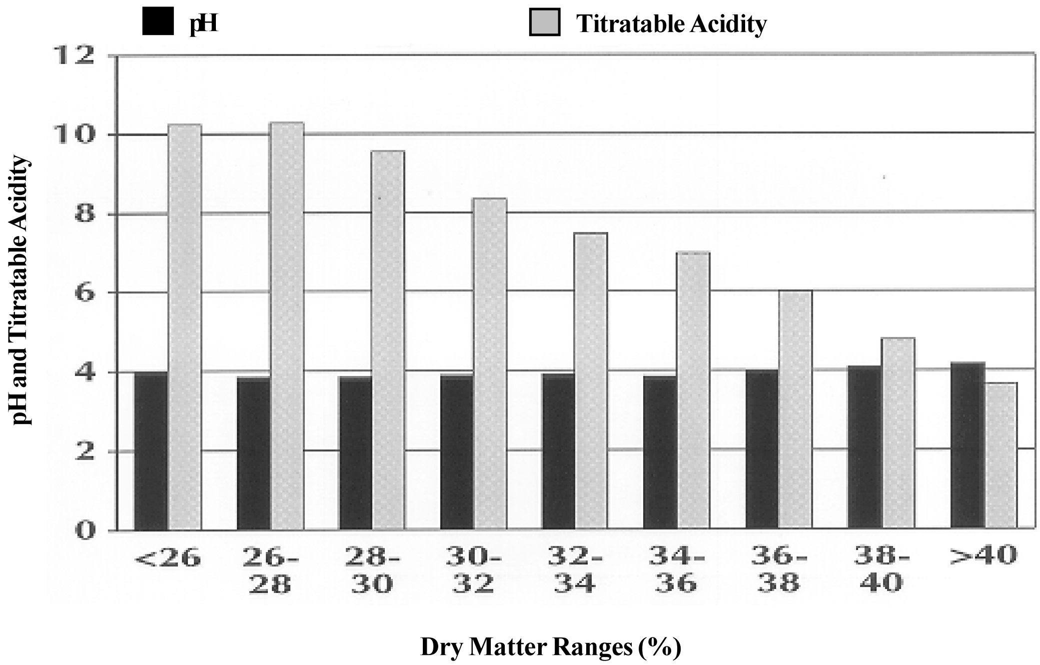

Figure 9. The pH and titratable acidity (meq/g) by dry matter range in corn silage.

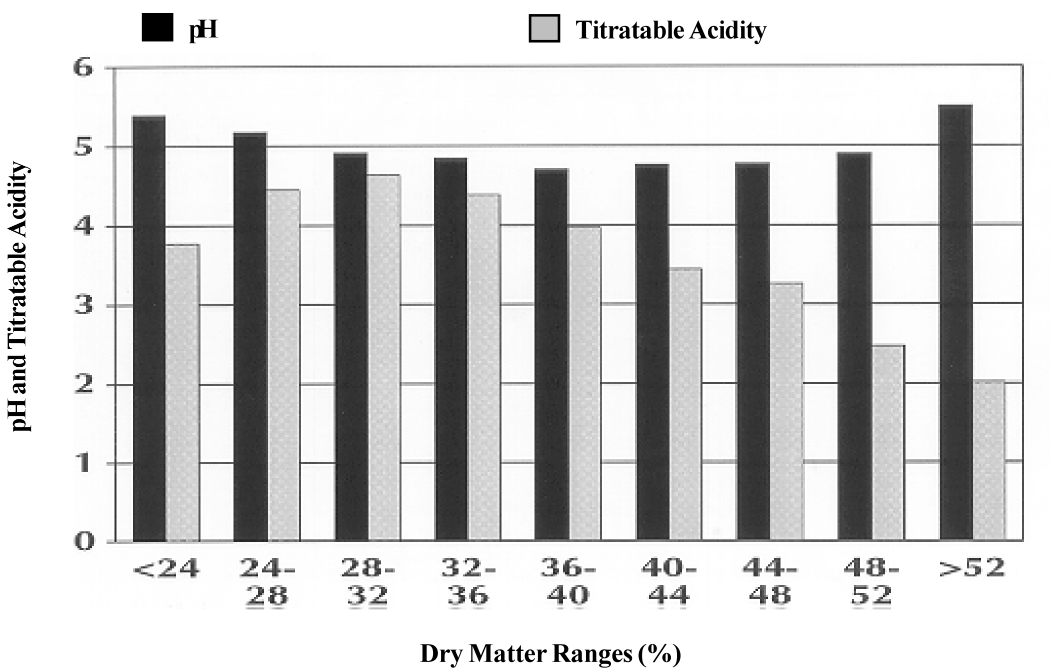

Figure 10. The pH and titratable acidity (meq/g) by dry matter range in legume silage.

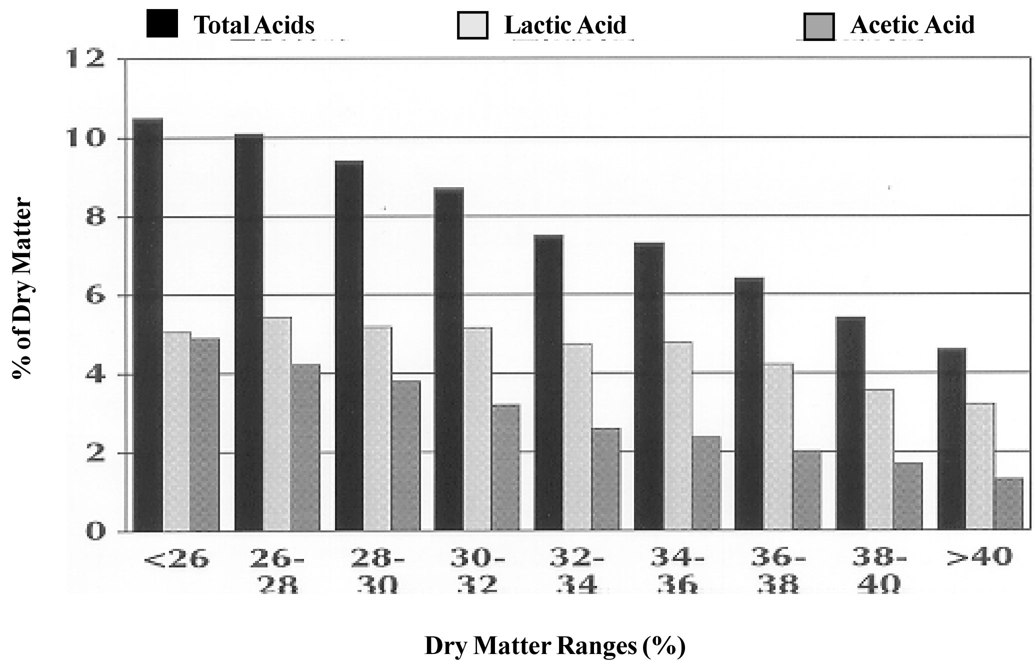

Figure 11. Fermentation acids by dry matter range in corn silage.

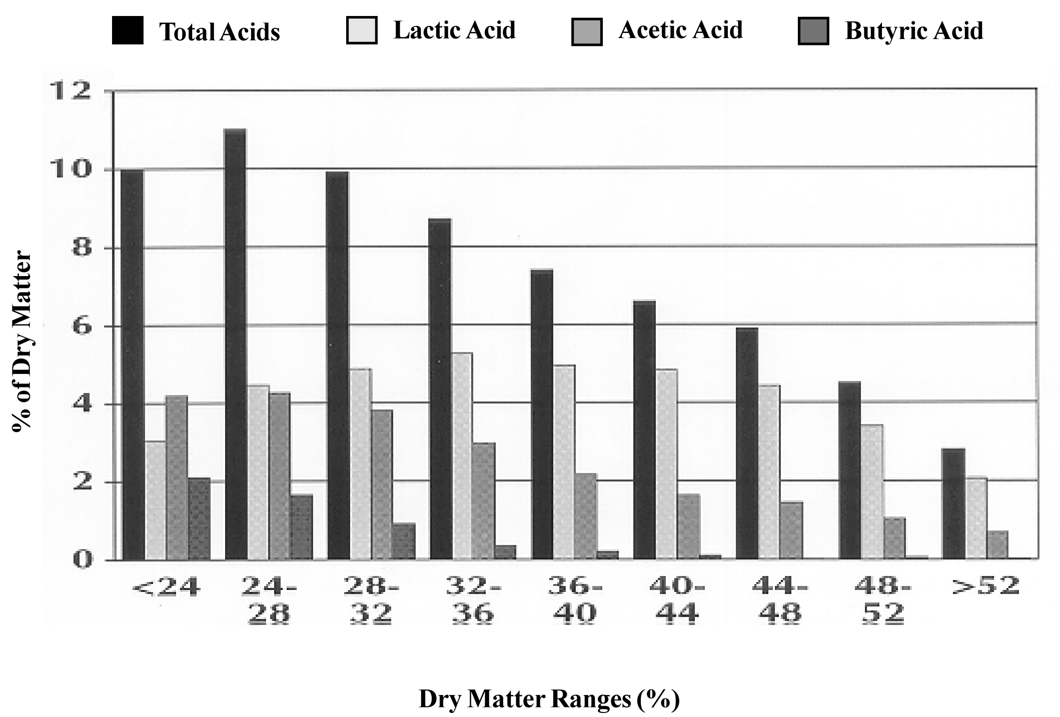

Figure 12. Fermentation acids by dry matter range in legume silage.