ABSTRACT

M.Sc. RICHARD FRANKLIN WELTON Nutrition

THE EFFICACY OF VARIOUS LABORATORY METHODS FOR THE EVALUATION OF FARM-PRODUCED FORAGES

Hay and grass silage samples were analyzed for protein and cellulose content, and Nutritive Value Indices (NVI) were predicted using the pepsin-HCl laboratory method. Estimated Net Energy (ENE) was determined for a sub-sample of 40 hays using Cornell Quality Codes, Penn. State, and Van Soest methods. First-cut hays were found to be different from second-cut hays due to the effect of plant species. ENE (Van Soest) and NVI were closely related due to a significant correlation ($r = .94$) between NVI and cell contents (Van Soest). Although ENE as determined by Penn. State - 3 formulas, NVI, Van Soest, and Cornell Quality Codes had similar means, the NVI values were distributed over a wider range. Sufficient variation of ENE content of the 40 hays as determined by all methods used (except Penn. State - 3 formulas) justified use of a laboratory method for evaluating hay. NVI was found to be an adequate method of determining the ENE content of Quebec first-cut hays. ENE content of 75 corn silage samples was observed to be relatively constant on a dry matter basis.

THE EFFICACY OF VARIOUS LABORATORY METHODS FOR THE EVALUATION OF FARM-PRODUCED FORAGES

by

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Hay and grass silage samples were analyzed for protein and cellulose content, and Nutritive Value Indices (NVI) were predicted using the pepsin-HCl laboratory method. Estimated Net Energy (ENE) was determined for a sub-sample of 40 hays using Cornell Quality Codes, Penn. State, and Van Soest methods. First-cut hays were found to be different from second-cut hays due to the effect of plant species. ENE (Van Soest) and NVI were closely related due to a significant correlation (r = .94) between NVI and cell contents (Van Soest). Although ENE as determined by Penn. State - 3 formulas, NVI, Van Soest, and Cornell Quality Codes had similar means, the NVI values were distributed over a wider range. Sufficient variation of ENE content of the 40 hays as determined by all methods used (except Penn. State - 3 formulas) justified use of a laboratory method for evaluating hay. NVI was found to be an adequate method of determining the ENE content of Quebec first-cut hays. ENE content of 75 corn silage samples was observed to be relatively constant on a dry matter basis.

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I. INTRODUCTION

A number of laboratory methods have been developed to measure the nutritive value of forages. Some of these methods involve one or more chemical analysis while other methods depend on _in vitro_ procedures utilizing rumen micro-organisms and/or digestive enzymes. Much of the developmental work in forage evaluation technique was carried out on a relatively small number of samples of pure forage species, rather than on a large number of samples of mixed species as generally produced on farms. Some of the laboratory methods for forage evaluation, although quite accurate when compared to _in vivo_ measures, were time consuming and hence could not be applied to large numbers of samples as required by a farmer-orientated forage evaluation service.

In May of 1966 a milk testing and herd analysis program was established by Macdonald College for Quebec dairy farmers. This computerized dairy herd milk testing program was the first of its kind in Canada and was called the Dairy Herd Analysis Service (D.H.A.S.). The service provided by D.H.A.S. for the dairy farmer is similar to that of Dairy Herd Improvement Association programs in operation in the United States. As part of the D.H.A.S. program all forages being fed to cows on test were evaluated, and on the basis of this evaluation and milk production measurement, grain ration recommendations were calculated for each cow. It was apparent that the accuracy of grain ration recommendations was dependent on the ability of the forage evaluation methods to accurately estimate nutritive value. Errors in forage evaluation could result in either inefficient use of grain ration from overfeeding or decreased milk production from underfeeding.

As a result of the establishment of the D.H.A.S. and plans of Macdonald College to establish a feed testing service the present study was initiated. The study herein reported had three objectives: 1) to determine if the present D.H.A.S. forage evaluation system using visual appraisal and tabular values could be improved by using a laboratory method; 2) to determine the effectiveness of techniques developed at Macdonald College to aid in computing feeding programs; 3) to compare several existing laboratory methods as to their effectiveness in evaluating the available energy content of hay, grass silage, haylage and corn silage so that the results can be utilized in a milk testing and feed recommendation program such as the D.H.A.S.

II. REVIEW OF THE LITERATURE

A. General considerations of evaluating farm-produced forages

1. Introduction

The search for methods which describe the "overall" nutritive value of feeds in numerical terms is continuing. Presently, several methods of evaluating forages and grain rations by the same criteria (e.g., TDN and ENE) are being used extensively in feed evaluation programs. When sources of readily available energy such as grains and their by-products are considered, the problem is less complex because possible sources of variation are not as great as with forages. The many forms of forage variation (e.g., variety and species differences, methods of curing, harvesting practices) present a much greater problem in regards to finding a meaningful quantitative description of their nutritive value.

In the nutrition of ruminants, the problem of feed evaluation is complicated by the complexities of ruminant digestive physiology and metabolism, which complicate the application of feed evaluation systems applicable to non-ruminants. This problem is also complicated by the fact that different forage species vary as to their nutritive contribution to the diet of the ruminant. However, certain factors are known to affect the nutritive value of forage. These factors may be environmental and/or "man-made." To effectively evaluate methods of measuring nutritive value of forage, some understanding of the influence of these factors on the nutritive value of forage is necessary.

Reid *et al*. (1959) wrote, "the main purpose served by forages in the diet of dairy cattle is the provision of energy." These workers outlined the concept of energy contribution by forage with the following scientific axioms:

a) size of animal response (milk yield, tissue maintained and gained) = energy intake;

b) energy intake = dry matter intake x energy concentration; and therefore,

c) forage energy intake = intake of forage dry matter x concentration of energy in forage dry matter.

Crampton *et al*. (1960) wrote, "with feeds of this category (forages) yield of energy to the animal is usually the first limiting factor in their feeding value since, in most cases when eaten as the entire ration, their nutritive content is adequate for the quality of the useful energy they provide." In accordance with the work of Reid et al. (1959), Crampton et al. (1960) and many other workers, the D.H.A.S. of Macdonald College evaluates feeds on the basis of available energy.

The various methods proposed for forage evaluation are also orientated toward measuring the energy contributions of the tested feeds. Therefore, this study is concerned with the effects of environment (natural - e.g. weather damage; "man-made" - e.g. date of cutting) as it may relate to methods of evaluating the energy content of forage.

B. Environmental factors as related to the energy content of forages

1. Stage of Maturity (date of cutting)

a. Timothy (*Phleum pratense*)

The loss of nutritive value of timothy due to delaying the harvesting date was clearly demonstrated by Mellin *et al*. (1962). Working with first-growth, pure-stand, climax timothy, Mellin *et al*. were able to show a decrease in protein content and protein digestibility at eleven stages of maturity, beginning on May 27, and at seven-day intervals until August 5. The decrease of protein content and digestibility of protein were more pronounced for each period of delay in harvest until July 22, when the values seemed to level off and remained fairly constant, probably due to the effects of bottom regrowth or possibly to seed formation. Coefficients of dry matter digestibility, decreased sharply with each delay in harvest, and a linear relationship was established between dry matter digestibility (Y), and days elapsing after May 17 (X), by the equation $Y = 84.91 - .481X$.

The effect of stage of maturity of timothy hay on its over-all nutritional value was measured by the Nutritive Value Index by Jeffers (1960). It was found that the Nutritive Value Index of timothy hay harvested on June 17 was significantly higher than that obtained for timothy after a fifteen-day interval and highly significantly greater than that for timothy hay harvested after intervals of twenty-nine days.

Four varieties of timothy, S-50 (*Phleum nodosum*), Climax, Drummond and Quebec Common (*Phleum pratense*) were compared by Heaney *et al*. (1966). These varieties were harvested at successive growth stages in 1962 and seven-day intervals until August 5. The decrease of protein content and digestibility of protein were more pronounced for each period of delay in harvest until July 22, when the values seemed to level off and remained fairly constant, probably due to the effects of bottom regrowth or possibly to seed formation. Coefficients of dry matter digestibility, decreased sharply with each delay in harvest, and a linear relationship was established between dry matter digestibility (Y), and days elapsing after May 17 (X), by the equation $Y = 84.91 - .481X$.

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Four varieties of timothy, S-50 (*Phleum nodosum*), Climax, Drummond and Quebec Common (*Phleum pratense*) were compared by Heaney *et al*. (1966). These varieties were harvested at successive growth stages in 1962 and

1963 from the same swards and were assayed for dry matter digestibility, dry matter intake and digestible energy content. These three indices of nutritive value declined from early vegetative stage through full bloom in a uniform manner for all varieties and rate of decline was not merely a function of time, but differed between seasons.

b. Orchardgrass (*Dactylis glomerata*)

The digestibility of freshly-cut green orchardgrass (S-143) was estimated with yearling heifers by Murdock *et al*. (1961). Digestibility of dry matter declined gradually from 75.6% at the start (April 23) to 74.0% at the preboot stage of growth (May 5), then falling rapidly to 60.0% at the full head stage (May 23) and then more gradually again to 54.8% at full bloom (June 6). Murdock suggested that on the basis of his data the relationship between dry matter digestibility and date of cutting could best be represented by a curvilinear relationship.

c. Alfalfa (*Medicago satira*)

The nutritive value of first cutting alfalfa, harvested at one-tenth bud, full bud and full bloom was studied with lactation and digestion studies by Davis and Decker (1959). Results of this study indicated a decrease in feeding value with increasing maturity, with a decrease of about 6% and 14% in milk production between material harvested at one-tenth bud and full bud, and one-half bloom, respectively.

Donker et al. (1968) compared different measures for hay evaluation on early-cut and late-cut alfalfa hays. For the early- and late-cut hays, respectively, the values on dry matter basis were: crude protein, 19.5 vs. 15.5%; crude fiber, 28.9 vs. 32.7%; range of TDN (%) 55 to 65 vs. 52 to 60. Estimated net energy (megacal. /45.4 kg. dry matter) 43 to 56 vs. 37 to 49. Holstein heifers used in this experiment consumed more early- than late-cut hay, expressed as percentage of body weight or per unit metabolic weight.

d. Species comparison

Orchardgrass (*Dactylis glomerata*), perennial rye-grass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and timothy (*Phleum pratense*) were used by Austenson (1963) to observe the influence of time of harvest on yield of dry matter and by digestibility predicted (Reid *et al.*, 1959). The hays were cut at three-week intervals from 21 April until 10 July, when all but timothy was in late bloom. Percentage leaf was inversely correlated ($r = -.89$) with yield of dry matter, however, there was little difference between species at the same stage of maturity. Protein was positively ($r = 0.96$) and lignin negatively ($r = -.94$) correlated with leafiness, and the negative correlation between lignin and protein was $r = -.95$. Timothy had more protein at the vegetative stage and less at the bloom stage, than did the other species. Yield of digestible dry matter calculated from equations based on leafiness or date of harvest (Reid _et al_. 1959) for all species was highest at full bloom.

Effect of early harvest on nutritive value of orchardgrass and timothy was studied by Brown _et al_. (1968). Digestibility of orchardgrass and timothy generally decreased with advancing maturity of the plants in spring. Dry matter intake of forage cut about May 20 was as high as that cut during the first week of May. Delaying harvest until June 12-13 reduced intake about 27%. Late maturing varieties of timothy and orchardgrass were slightly higher in dry matter digestibility than early maturing varieties cut at the same date. Intake was similar for the two varieties within each species, however, forages harvested on comparable dates were more digestible in 1963 than in 1964. Dry matter (DM) and digestible energy (DE) were closely related to leaf percentage (L) and protein content of the forage. From these relationships the following equations were developed: $DE = 48.8 + 0.35L$ and (digestible dry matter) $DDM = 0.36L + 51.9$. Brown et al. (1968) observed that leaf content appeared to be a better indicator of digestibility than harvest date, however, determining leaf percentage would be a much more laborious procedure.

Influence of cutting date upon forage intake was investigated by Reid et al. (1959). These workers observed that forages (mixed hays) cut during early June were consumed at the rate of 2.5 to 3.0 lb. of hay equivalent per 100 lb. body weight, while hays harvested during mid-July were consumed at 1.1 to 1.7 lb. per 100 lb. body weight. These workers also pointed out the inadequacy of TDN as a measure of forage quality when the forage was fed ad libitum, since intake was not considered in the TDN system of evaluating forages. A significant relationship between per cent digestible dry matter (Y), and days cut after April 30 (X), was established as represented by the equation $Y = 85.0 - 0.48X$. This equation did not apply to aftermath or to first cut hay harvested after July 12. Later work by Mellin et al.

(1962) with timothy hay showed a similar relationship between digestible dry matter (DDM) and days of cutting after May 17. Other equations for calculating DDM were presented by Reid *et al.* (1959). These equations were: DDM = 0.4L + 40.8, and DDM = 87.4 - 1.042X, where L = percentage leaf content and X = dry matter content of forage as harvested.

A series of feeding trials conducted over a five-year period by Slack *et al.* (1960) clearly demonstrated the superiority of early-harvested forages in promoting and sustaining high milk production. The difference in milk production favoring the early-cut forages was more marked in the 20-week continuous feeding trials than in the 5-week change-over design feeding trials.

2. Effect of Cuttings

Researchers have observed that aftermath hays (hay cut after the first cutting) seem to differ from first-cut hay. Reid *et al.* (1959) established a relationship between digestible dry matter (DDM) and days after April 30 but suggested that all aftermath hays had a DDM between 57% and 64%, and none contained as much DDM as first cutting harvested before June 10. The equation whereby DDM was calculated from leaf content did not apply to aftermath hays.

The nutritive value of timothy hay at different stages of maturity as compared with second cutting clover hay was studied by Colovos _et al_. (1949). These workers observed that early-, medium- and late-cut timothy hays contained 57.1, 44.1 and 32.5%, respectively, as much protein as the second cutting clover. However, gross energy values for all the hays were essentially the same. The digestibility of the protein decreased markedly from the clover hay when the different timothy hays were compared to clover hay, with early, medium and late timothy hays furnishing only 49.7, 31.5 and 15.4%, respectively, as much digestible protein as was furnished by the clover hay.

Early-cut timothy hay was superior to the other hays with respect to metabolizable energy as determined by dairy heifers in an open-circuit respiration chamber. These workers suggested that the results of their experiments showed that early-cut timothy hay may be a better source of energy than good legume hay for dairy cattle but not of digestible protein.

In vitro dry matter digestibility, water soluble carbohydrates, crude protein and mineral content of six cuts of clover and lucerne were used by Davies *et al*. (1966) to evaluate the effects of different cuttings. Average dry matter when cut was 14.5% in the first cut and rose fairly steadily to 26.3% in the sixth; average *in vitro* digestibility of dry matter declined from 78.7% for the first, to 63.1% for the sixth cut. Crude protein decreased from an average of 26.7% for first cut to an average of 14.5% for the sixth cut. Water soluble carbohydrates reached an average of 11.4% in white clover, 9.9% in red clover, and 7.2% in lucerne; there was some difference due to cut but not species. Correlations of age of herbage with percentages of dry matter content when cut, crude protein and dry matter digestibility were highly significant as discussed in the preceding subsection and were in agreement with the *in vivo* work of Reid *et al*. (1959) and Mellin *et al*. (1962).

At Macdonald College Donefer and Lloyd (1955) compared first cutting and aftermath hays in ground and chopped forms. They observed that the digestible energy coefficients of aftermath alfalfa and timothy hays were 2 to 7 percentage units higher than the first cut hays. However, except for chopped timothy aftermath, the relative intake of the other aftermath hays was 2 to 8 percentage units lower than first cutting hays.

Nutritive Value Indices (digestible energy x relative intake) for both alfalfa and timothy aftermath hays generally increased when compared to first cuttings.

3. Methods of Curing

a. Field cured vs. barn dried hay

Turk (1952) indicated that the greatest advantage of barn drying appears to be the preventing of dry matter losses that normally occur with field curing. In a majority of the experiments reported, barn drying preserved from 81% to 85% of the dry matter harvested compared to 63% to 80% with field curing.

According to Turk's report, protein losses varied from 20% to 24% for barn-dried hay, with the lower percentage where supplemental heat was used. In comparison, protein losses from field curing varied from 28% to 46%, depending upon weather damage before the hay was stored. Protein losses in the mow are greater with barn-dried hay than with field-cured hay. Turk concluded that although barn-dried hays, based on greener color, more leafiness and protein in the case of legumes, are usually higher in quality, there has been no appreciable difference in feeding value on per ton basis as fed. However, because of the saving in dry matter resulting from barn drying there is a difference favoring barn drying on a milk production per acre basis.

Barn drying hay at high temperatures can affect the nutritive value of hay, as was observed by Bratzler *et al.* (1960). Four lots of alfalfa harvested under similar conditions were dried at temperatures of 110°, 135°, 165°, or 200°F. for 20, 12, 7½ and 5½ hours, respectively. The hays were then fed to sheep in a digestion trial. Crude protein, dry matter and gross energy were slightly less digestible in hay dried at the extremes of 200°F. or 110°F. In the hay dried at 110°F. a low level of nitrogen-free extract indicated that respiration by the plant had continued, or fermentation had occurred at that temperature.

Ekern *et al.* (1964) investigated the effect of artificial drying and freezing on the energy value of pasture herbage. These workers were able to show that artificial drying at 100°C. depressed the metabolizable energy of herbage by 4%, which was in agreement with Bratzler *et al.* (1960). Artificial drying, however, significantly increased the efficiency of utilization of the metabolizable energy of the herbage by 9%. Thus, the net energy as a per cent of the gross energy was increased by 5% as a result of artificial drying.

Results of storage by freezing followed the same pattern but were not as pronounced. Ekern and associates concluded that the increased net energy level was due largely to smaller losses of heat and carbon dioxide, when the metabolized energy is derived from preserved herbage rather than from fresh herbage.

b. Silage vs. barn-dried hay

An experiment lasting two years, using 16 bullocks the first year and 24 in the second year, was reported by Culpin (1962) in which the feeding value of silage and barn-dried hay was compared. As silage, the same crop had less loss and the material had lower crude fiber and higher starch equivalent (net energy contents) on a dry matter basis. With hay the digestibility of dry matter and over-all performance of the bullocks given hay was better.

When wilted silage and barn-dried hay were compared by Howie (1964) the results were not in agreement with Culpin (1962). Forage of similar botanical composition was made into hay by barn drying or ensiled after wilting. Yields of starch equivalent (SE) per acre were the same for the two fields studied, but because of the earlier cutting of silage, yield of SE in the aftermath on that field was six times greater, indicating the superiority of silage in the economic use of land. According to the work of Howie (1964) cows given the silage produced significantly more milk and gained more liveweight than those given hay. The difference between the results of Culpin (1962) and Howie (1964) may have been due to different dry matter contents of the silages.

c. Silage vs. field cured hay

The nutritive value of silage as compared with hay was investigated by Brown et al. (1963). Silage and hay were made from alfalfa harvested at the same stage of maturity from the same field. Rations were all hay, all silage, or hay:silage mixtures in 75:25, 50:50, or 25:75 proportions. These rations were fed in two separate trials to 5 groups of 5 Holstein cows. In a third trial hay and silage were given separately with and without limited amounts of concentrate. Results of the first two trials indicated that intake of dry matter per 100 lb. body weight was significantly higher for rations with 50% or more hay than when only silage was fed. Yield of fat corrected milk was higher in the groups given 50% or more hay. When grain was given there was a significant decrease in intake of dry matter from hay and silage but the total intake of dry matter increased. Concentrates increased milk yields more with silage than with hay and these differences were significant. Brown and co-workers suggested that in addition to the appetite depressing factor in silage, as indicated by low dry matter intake at high levels of offered silage, there may be other factors (in silage) which stimulate milk production.

Wellmann (1966) compared the dry matter intake of silage and hay from the same crop. These findings were in agreement with Brown et al. (1963) and showed that animals tended to consume larger amounts of hay than silage on a dry matter basis.

The work of Brown et al. (1963) and Wellmann (1966) agrees with the earlier work of Slack et al. (1960) on the dry matter intake of the different types of forage. This experiment revealed that cows fed early harvested grass silage consumed less dry matter per day than cows on barn-dried hay cut at the same time, or late field-cured hay. However, in contrast to the work of Brown et al. (1963), more milk was not produced on rations containing more than 50% hay. According to

Slack *et al*. (1960), significantly more milk was produced on early silage than on early barn-dried hay, based on dry matter intake. Body weight and condition were maintained better on early silage-fed cows than on those receiving early barn-dried hay or late field-cured hay. The more efficient utilization of silage dry matter would appear to be due to a higher digestibility on the early harvested silage. Also milk production was not calculated on a dry matter intake basis by Brown *et al*. (1963) or Wellmann (1966).

In a subsequent experiment Slack and associates compared the same forages under two levels of grain feeding (10 and 6 lb. per day). This experiment indicated that dairy cows cannot consume enough dry matter from roughages, whether early-cut or late-cut to compensate for a reduction of grain-feeding levels. However, cows will produce more milk on a low grain level and early-harvested forages than they will on higher levels of grain and late-cut forages, thus demonstrating the grain-saving power of early-harvested forages.

The question of relative feeding values of alfalfa hay, silage and low-moisture silage (haylage) was answered by Byers (1965). Alfalfa was cut from the same field and made into hay after drying for 48 hours, or silage after wilting to moderate or high dry matter content. The forages were fed to appetite for 56 days, with concentrate fed according to milk yield, to groups of 5 Holstein and 5 Brown Swiss cows. Digestibility was determined by the Cr_2O_3 method. The dry matter of hay, haylage and silage was 86%, 50% and 24%, respectively. When dry matter intake, milk yield, liveweight change, milk produced per lb. dry matter of forage eaten, and digestibility of nutrients were used as criteria, no difference between alfalfa stored as hay, haylage or grass silage was observed.

One of the problems, from the farmers' point of view, in making grass silage has been the production of highly objectionable odors. This problem was investigated by Trimberger _et_ _al_. (1955) by comparing three silage preservatives. Over a 2-year period the average seepage losses were 7.4, 4.4, 7.4, and 6.0% for molasses, brewers grain, no preservative, and sodium bisulphate, respectively. Fermentation losses of the same silage were 15.4, 15.2, 15.2 and 16.0%. All silages were ensiled at 80% moisture, which was higher than the 75% moisture level normally observed for grass silage. It was concluded by Trimberger and associates that the small saving of nutrients which resulted from the use of preservatives did not justify the high cost of the preservative. However, if conditions on the farm were such that silage without objectionable odors could not be made without using a preservative, then the farmer may be willing to pay the higher cost of silage made with sodium bisulphate for the sole purpose of reducing odors.

d. Specialized methods of curing forages

A number of more specialized methods of curing have been tried experimentally, but only a few of these methods have been suitable to practical farm situations. Due to portable and stationary hay dryers, heat dehydrated forages, particularly alfalfa have become more widespread. The effect of dehydration of alfalfa hay was investigated by Meyer _et al_. (1960). A four-year study of weight gains and feed consumption of lambs indicated that dehydrated alfalfa was superior to field-cured alfalfa as an energy source in all cases.

4. Effect of fertilization

The effect of fertilization on the nutritive value of forages has been investigated by a number of researchers; Burton and DeVane (1952), working with Bermuda grass hay; Reid and Jung (1965a), with tall fescue hay; Mosi (1967) used mixed herbage; Cameron (1966), with grass forage. These workers have made similar observations that nitrogen fertilization significantly increased crude protein and digestibility of protein while also increasing crude fiber content and yield of dry matter.

In a symposium on forage utilization, Blaser (1964) summarized the effects of fertility levels on forage nutritive value. He pointed out that nitrogen fertilization of grasses has given large increases in carrying capacity and livestock products per acre, but outputs per animal unit are not generally improved by nitrogen application. According to Blaser (1964) nitrogen fertilization improves protein content and its apparent digestibility, but that cellulose or crude fiber content, and lignification are not generally altered. This researcher suggests that, due to a reduction in soluble carbohydrate and digestible protein for energy resulting from nitrogen fertilization, there is not an appreciable change in TDN or digestible energy content of these forages.

More pronounced improvement of the nutritive value of grass hays was observed by Markely _et al._ (1959) and Chalupa _et al._ (1961) than was indicated by other investigators. Markely _et al._ (1959) found that the addition of 200 lb. of nitrogen per acre to bromegrass and orchardgrass not only increased crude protein and digestibility of protein, but also increased digestibility of fiber, and gross energy which produced increased TDN. The work of Chalupa _et al._ (1961) with reed canary grass and alfalfa revealed similar improvements.

5. Weather Damage

Minimizing forage nutritive losses caused by exposure to weather has long been considered a major factor in making high quality forage but literature references to the effect of weather are relatively hard to find. Maymone (1952), in a series of four experiments in Southern Italy, discovered that the fall of 25 mm. of rain caused losses equal to 43.28% of the dry matter and 47.72% of net energy (expressed in starch units) and 49.61% of the digestible protein. Losses due to rain were greatest at the latter part of drying. It would appear that at the latter stages of drying the leaves were more brittle and were easily broken off by the rain.

Various methods of harvesting and preserving forages and rain damage were investigated by Moore and Shepherd (1952). These workers observed greater losses of dry matter, protein, and carotene occurred with field curing under excellent weather conditions than with barn curing or wilted silage. Still greater losses occurred with field curing when the crop was rain damaged. Feeding tests by these workers showed that the forages cured by the various methods were all about equal in feeding value except the rain-damaged, field-cured hay which produced a 13.6% decline in milk yield compared to 7.3% for wilted silage.

In two years of trials to determine the rate of loss of nutrients in hay, Murdock and Bare (1963) were able to observe the effects of rain. When the hays were sprinkled with water to simulate rain, the dry matter digestibility of untreated hays was not much affected but the digestibility was reduced in the case of the crimped hay. Evidently the effects of conditioning (crimping) promoted leaching losses which adversely affected digestibility.

Digestibility of dry matter by sheep was the criterion used by Coetzee (1966) to differentiate the quality of sun-cured and rained-on lucerne hay. This work demonstrated that the intake of coarsely ground hay per unit metabolic weight was the lowest for the rained-on hay.

The development of a prediction equation for *in vivo* digestible dry matter from *in vitro* digestible dry matter by Barnes (1966) made it possible for the simultaneous evaluation of both grasses and legumes. From prediction equation developed by Barnes, dry matter digestibility (DMD), Relative Intake (RI) and Nutritive Value Index (NVI) were predicted for second-cut alfalfa raked before and after rain. The percentages of DMD, RI and NVI for the dry hay were 52.3, 79.3 and 48.0, respectively, while the values for the rained-on hay were 49.9, 70.1 and 43.9, respectively. The detrimental effects of rain as measured by DMD, RI and NVI are evident with RI and NVI being most adversely affected.

6. Morphology of plant

The reduced digestibility of different structural parts of plants was observed by Mowat *et al.* (1965). They found that the *in vitro* dry matter digestibility curves of timothy, orchardgrass, bromegrass, and alfalfa were similar for each year. At head emergence the *in vitro* dry matter digestibility of the leaves and stems of all grasses was somewhat similar but wide differences existed in the digestibility of the leaves and stems of alfalfa at all stages of growth. As plant species matured there was a decrease in digestibility of both leaves and stem. Digestibility of stems was lower for all species after head emergence and the slope varied with the individual species.

Rony (1964) determined that the *in vitro* cellulose digestion for both alfalfa and brome grass was higher in the leaf than in the stem fraction due to the presence of a larger lignin content in the stem.

Palatability of plants as affected by leaf flexibility was studied in tall fescue by Gillet and Jadas-Hecart (1965). Experiments with grazing of spaced plants showed important genetic differences due to leaf flexibility, which could be determined by touch. This characteristic did not depend upon chemical composition or some particular characteristic of leaf cells, but probably on the size of leaf fibrovascular bundles.

Information of the nutritive value of plant parts for several species can be found in some of the more recent feed composition tables. An example of available data for alfalfa is shown in Table 1.

TABLE 1.--Composition of alfalfa plant parts on a dry matter basis (joint United States-Canadian tables of feed composition)

Plant part	Protein	Crude Fiber	TDN	Digestible Energy
	%	%	%	kcal./kg.
Leaves (all analysis)	24.0	16.4	65	2866
Stems (all analysis)	10.7	44.4	46	2028
Whole plant (all analysis)	17.3	31.4	56	2469

7. Plant Species

It is well known that many plant species differ greatly in crude protein and crude fiber content, whereas the differences in energy content are not as pronounced and are more dependent on stage of maturity (Reid *et al*. 1959). However, there are other species differences which are not so well established. Maymone (1952) discovered a large difference between alfalfa and mixed herbage as to losses of nutritive value (net energy), dry matter, and digestible protein. These losses were more pronounced for alfalfa than for the mixed herbage.

Species differences of trefoil, clover, brome, timothy, and straw, as revealed by digestible energy intake, percentage composition of protein, crude fiber, and lignin, were observed by Crampton (1957).

Nutritive Value Index (NVI) was studied _in vivo_ and _in vitro_ by Donefer _et al_. (1960). These researchers, working with alfalfa, red clover, birdsfoot trefoil, bromegrass, and timothy, observed that the NVI varied with species, indicating that legumes were consistently higher than grasses. Similar differences between legumes and grasses, as determined by NVI, were observed by Lloyd _et al_. (1960). Lloyd and associates demonstrated that red clover had a significantly higher relative intake and NVI than timothy.

Differences in the digestibility of alfalfa hay and reed canarygrass were established by Archibald _et al_. (1962) using four cows in a digestion trial. Crude fiber, cellulose, and pentosans of reed canarygrass were better digested by the cows. However, the digestibility of energy was the same for both hays.

Kivimae (1966), while studying the digestibility and feeding value of timothy, discovered that the carbohydrate fraction in timothy behaved differently to legumes during the growing period. The content of crude fiber increased curvilinearly (convex) with a maximum at full flowering. The digestibility of nutrients and feed value decreased successively in the corresponding period. Crude fiber was found to be an unreliable substance for estimating feed value, particularly for stages after heading, while lignin and methoxyl gave a better estimation.

Digestibility differences between perennial ryegrass, meadow fescue, Italian ryegrass, timothy, and cocksfoot were small but consistent according to Dent and Aldrich (1966). These workers determined digestibility by in vitro over a 2-year period and concluded that differences were dependent on management and species.

Nitrogen-fertilized orchardgrass was compared with alfalfa at different levels of concentrate by observing their effect on milk production. This work by Apgar et al. (1966) demonstrated that more milk was produced from alfalfa, and was probably due to the greater intake of alfalfa.

C. Chemical methods for evaluating the nutritive value of hay and grass silage

1. Proximate analysis

German workers (Henneberg and Stohmann 1864), working at the Weende Experimental Station, developed a scheme of fractionating feedstuffs into the "proximate principles": crude protein, crude fiber, ether extract, ash and nitrogen free extract. Although a chemical rather than a biological measure, several of the proximate principles have been studied as possible predictors of the following biological measures of forage nutritive value.

a. Digestible protein

Holter and Reid (1959) worked with data from Cornell and data from Morrison's Feed and Feeding (1956) to develop a mathematical relationship between digestible protein and crude protein. These workers found the Cornell data, obtained over 8 years of digestion trials with sheep and cattle and involving 27 fresh, green forages and 20 hays, gave a reliable prediction equation. This prediction equation had a low standard error of the estimate of 0.46% and was applicable to all types of forages and equally expressed the relationship between crude protein and digestible protein in leaves and stem of alfalfa as well as grass fertilized at high nitrogen levels.

Elliot and Fokkema (1960) used tables in Schneider's book Feeds of the World to determine the relationship between digestible protein and crude protein as observed from 388 cattle and 895 sheep feeds. An equation was established for calculating digestible protein for cattle, while a slightly different equation was established for the same calculation for sheep and goats.

Forage crude protein was observed by Baumgardt *et al*. (1962) to be highly correlated ($r = 0.999$) with digestible protein. A similar association between crude and digestible protein was observed by Stallcup and Davis (1965).

Digestible protein of forages was successfully calculated from crude protein by Dijkstra (1966). The relationship between digestible protein and crude protein did not vary significantly from crop to crop. The calculations of Dijkstra were made expressing protein on an organic matter basis rather than on a dry matter basis.

b. Digestible energy criteria

In a review on evaluating the nutritive quality of forage on the basis of energy, Hardison (1959) states that digestible dry matter (DDM), total digestible nutrients (TDN), and digestible energy (DE) are all essentially a measure of the same entity. Hardison indicates that these common measures of nutritive value represent the difference between feed consumed and feces voided. He further states that since DDM, DE and TDN are representative of the proportion of ingested matter or energy that disappears as the feed traverses the gastrointestinal tract, they are highly correlated, one with the other. These high correlations were also observed by Swift (1957), Heaney and Pigden (1963), and Stallcup and Davis (1965).

A number of investigators have contributed methods of determining TDN from chemical analyses (proximate analysis). One of the first was Schneider *et al*. (1952). These workers developed an equation to predict TDN from the combination of crude protein, crude fiber, nitrogen-free extract and ether extract. They found that ether extract could be omitted from the prediction equation with very little loss of accuracy.

Axelsson (1952) produced an equation for calculating TDN from digestible protein and crude fiber content of forages.

A prediction equation with a standard error of the estimate of 2.52 for predicting TDN from crude fiber plus silica was developed by Meyer and Lofgreen (1959). The standard error of the estimate for this equation was slightly less than a similar equation predicting TDN from crude fiber but these relationships only applied to alfalfa.

The TDN of all forages could be predicted from the equation of Glover et al. (1960). These workers predicted TDN from crude protein and crude fiber but TDN values were reduced more than expected when the protein content of the forage was below 5%.

Baumgardt et al. (1962) estimated TDN from digestible protein and crude fiber. These values were found to be significantly correlated with in vivo digestion coefficients for dry matter, organic matter and energy but were not significantly correlated with any animal criteria studied.

Work by Adams et al. (1964) indicated that separate prediction equations for calculating TDN from crude protein and crude fiber content were necessary for each species of forage.

c. Estimated net energy (ENE)

Researchers have not been able to successfully predict ENE directly from proximate analyses. However, through the work of Moore et al. (1953) an indirect calculation was made possible. Moore and associates developed a prediction equation to predict ENE from TDN. Although the relationship was different for legumes and grasses, the results for the two species groups were combined to produce a single prediction equation.

Kane (1962) used ENE and TDN values from Morrison's Feed and Feeding (21st and 22nd edition) to develop an equation for predicting ENE from TDN. The data for 225 combined feedstuffs (21st edition) were utilized to develop one formula while data from 273 feedstuffs (22nd edition) were used to develop the second equation. These two prediction equations were very similar due to much data in both of Morrison's editions being similar.

2. Other chemical analyses

A number of chemical analyses, other than the proximate analysis have been used to predict *in vivo* criteria that measure the nutritive value of forages.

a. Digestible energy criteria

Forbes and Garigus (1948) found that TDN (a measure of digestible energy) could be predicted from lignin content. These workers used nine samples of ladino clover, orchardgrass, Kentucky fescue, and Kentucky bluegrass to conduct a digestion trial with sheep. These workers reported that TDN determined from the trials had a high inverse correlation with lignin content of the forages.

Common (1952) pointed out that digestible organic matter (a measure of digestible energy) was highly correlated ($r = -.978$) with lignin for a group of New Zealand forages. However, the same relationship for Irish forages was greatly reduced ($r = -.419$). Common suggests that this difference should make the researcher aware that regression relationship can vary from one set of data to the next, and means that a researcher must choose the more reliable relationships, which applies to the data he is working with.

Work by Sosulski and Patterson (1961) revealed highly significant negative correlations between digestible energy (DE) and lignin content. These workers used 6 species of grasses each harvested in 2 different years and determined the correlation coefficient between DE and lignin for 1956 and 1957 to be -.903 and -.948 respectively. Very little improvement in the correlation relationship was observed by Sosulski and Patterson when DE was compared with both the crude protein and lignin content of the forages.

High inverse correlation between digestible dry matter and lignin content of forage was reported by Mohammed (1966).

Another approach to determining the digestible dry matter (DDM) was taken by Anthony and Reid (1958). This approach was based on the fact that since lignin contains methoxyl groups (Phillips 1940) measurement of methoxyl content of forages should indicate indigestibility. Three forage mixtures of different botanical composition and harvested at several stages of growth were used to determine the relationship between DDM and methoxyl content of forages. These workers showed a highly significant negative correlation between DDM and methoxyl content ($r = -.83$) for forage and suggested that this relationship could be used in predicting DDM. However, this relationship was not as high as the relationship between digestible energy and lignin as obtained by Sosulski and Patterson (1961). Another limitation is the use of methoxyl is the fact that lignin content can be determined more easily than methoxyl content.

b. Net energy and ENE

A relatively simple and inexpensive method for determining net energy was proposed by Armstrong et al. (1964). These researchers worked with 16 dried forages to establish an equation to predict ENE from lignin. A high residual standard deviation of 13% was observed when all the herbages were used to predict ENE for fattening from lignin. However, when the herbages were classified into rye-grasses and other grasses the residual standard deviation of predicted ENE was reduced to 4.8%.

c. Digestible laboratory nutrients (DLN)

Thurman and Wehunt (1955) developed a laboratory method of digesting forage with concentrated HCl. They found that DLM were significantly correlated ($r = 0.66$) with TDN but this relationship was not high enough to develop an accurate prediction equation.

When Baumgardt *et al*. (1962) compared DLN with TDN, digestible organic matter, digestible dry matter and digestible energy the correlation relationships were not significant. This work was not in agreement with the work of Thurman and Wehunt (1955) and a possible explanation was that Baumgardt and associates worked with hays while Thurman and Wehunt used sorghum silage.

d. Voluntary Intake (VI)

Intake of forages as related to nutritional value of forages has been discussed by Reid *et al*. (1959), Crampton (1957), McCullough (1959), and Conrad *et al*. (1964). Experiments at Macdonald College by Lister (1957) enabled him to develop a multiple regression to predict forage voluntary intake. In this equation voluntary forage intake (gm./day) was predicted from body weight (lb.), percentage crude protein content of forage, percentage crude fiber content of forage and phosphorus content of forage (mgm./g.).

Only two chemical fractions were found to bear significantly on voluntary intake by Smith (1958). Voluntary intake was found to be directly correlated with protein content and inversely correlated with cellulose content. Although these relationships were highly significant, it was impossible to account for more than 59% of the variability in voluntary intake by chemical composition of forages.

Van Soest (1965b) investigated the factors influencing voluntary intake in relation to chemical composition and digestibility. He found that chemical composition on the whole is much more closely related to digestibility than voluntary intake. In some forages (orchardgrass, bromegrass, and Sudan grass) the relation between voluntary intake and chemical components was very high and could be predicted with some accuracy.

Chemical analysis was used by Johnson _et al._ (1965) to predict voluntary intake (Relative Intake). Johnson and associates found that the solubility of forage in 1.0 N H_2SO_4 could be used to predict Relative Intake. Similar work by Mohammed (1966) showed that voluntary intake and forage solubility in H_2SO_4 were highly correlated ($r = 0.99$).

3. Detergent Analysis (Van Soest)

When Thaer (1809), Henneberg and Stohmann (1860), originally prepared crude fiber it was their belief that this fraction represented the indigestible portion of feedstuffs. However, additional work by these workers revealed that in some feedstuffs (forages) crude fiber was better digested than nitrogen-free extract. Since the discovery of the digestibility of crude fiber, numerous attempts have been made to find a method whereby feedstuffs could be divided into digestible and indigestible fractions.

In recent years a most comprehensive study of chemical methods to fractionate plants into constituents related to animal performance has been conducted under the direction of P. J. Van Soest, working at the Beltsville, Maryland laboratory of the United States Department of Agriculture. The first approach was to find an easier method of determining lignin. Van Soest (1963) reviewed chemical methods of analyzing for lignin and then investigated reagents for digesting forages. He found that sodium laurel sulfate in neutral or slightly alkaline solutions (neutral detergent) and cetyltrimethyl ammonium bromide in strongly acid solutions (acid detergent) could be utilized to divide forage into fractions which were either uniformly digested or not digested by ruminants.

A new method for the determination of fiber was published by Van Soest (1963). In this paper the method of determining acid-detergent fiber (ADF) and the subsequent treatment of ADF by cold 72% H_2SO_4 to produce acid-detergent lignin was outlined. Van Soest points out that ADF is representative of the fibrous portion of forage and was more highly correlated with digestibility data than was crude fiber.

Neutral detergent was used by Van Soest and Wine (1967) to fractionate forage material into a soluble portion and an insoluble portion which the latter termed neutral-detergent fiber and was composed of plant cell walls. The soluble portion was representative of the soluble portion of the plant cell and was found to be 98% digestible.

In an effort to eliminate any factors, which might affect digestibility, Van Soest (1965c) investigated the effects of drying forage before chemical analysis. His observations were in agreement with the work of Bratzler et al. (1960), and Ekern et al. (1964), who observed decreases in digestibility of crude protein, dry matter, and gross energy due to drying. Van Soest (1964) attributed this reduction of digestibility to an increase in artifact lignin and consequently recommended that forage samples not be dried at a temperature above 45°C.

The application of cell-wall constituent and cell-wall content analysis to evaluate the available energy content of forages was discussed by Van Soest and Moore (1965). Digestibility of cell-wall constituents was shown to be controlled by the concentration of lignin in lignocellulose. The cell contents were shown to be highly digestible and unaffected by lignin. This indicated to Van Soest that one chemical fraction could not accurately be used to predict the nutritive value of forages. Consequently equations were developed utilizing lignin, acid-detergent fiber, and cell contents to predict percentages digestible dry matter, digestible energy and TDN.

A number of problems were encountered by Van Soest when he tried to determine the true digestibility of cell walls. These problems were discussed by Van Soest (1967).

During the last 5 years a number of researchers have utilized Van Soest's methods in their work. Clancy and Wilson (1966) slightly modified Van Soest's (1963) method of determining acid-detergent fiber and contrary to Van Soest's suggestion, used his modified acid-detergent fiber to establish a high correlation with *in vivo* dry matter digestibility (r = -.85). Gaillard (1966), utilizing a number of chemical fractions, discovered a high multiple correlation (r = 95) between digestibility of organic matter and forage content of acid detergent lignin, cellulose, hemicellulose, and anhydro-uronic. Although the correlation coefficient was high and residual standard deviation was low for this relationship, the laboratory procedures involved in the chemical analysis may be too involved for handling large numbers of forage samples.

The chemical composition of the cell-wall constituents (CWC) and acid-detergent fiber (ADF) fraction of forages were investigated by Colburn and Evans (1967). These workers using 7 lucerne and mixtures and 14 grasses were able to show no species difference in cellulose content of ADF (mean value 79%) but the lignin content of ADF for grasses (10.6%) was significantly less than that of the lucerne and mixtures, which was 16.3%. Summation of cellulose, lignin, crude protein, and ash accounted for 95% of the total ADF for grasses and 99% for lucerne and mixtures. The ADF analysis recovered

92% of the plant cellulose and 6% of the crude fiber. Cellulose contents (C) could be predicted from ADF minus lignin (c) by the equation $C = 4.56 - 0.81c$ for grasses and $C = 5.66 - 0.83c$ for lucernes (alfalfa) and mixtures. On the basis of this work Colburn and Evans (1967) agreed with Van Soest that ADF was a more precise entity than crude fiber and suggested that acid-detergent lignin may represent a more hard-core lignin.

4. Mechanical Methods

It appears from the literature reviewed that very little work has been done to utilize some mechanical method to evaluate the nutritive value of forage. Probably the first step in this direction was taken by Troelsen and Bigsby (1964). These workers converted a double gear pump into a masticator, which was used to masticate 14 hays that had been fed to sheep. After mastication the hay samples were dried and passed through a series of sieves to determine a particle size index. Troelsen and Bigsby were able to establish a high correlation ($r = 0.94$) between forage intake and particle size index.

More recently, Chenost (1966) developed a method of assessing the degree of fibrousness of hay from the measurement of the electrical energy required to pulverize a 5-gm. sample of hay. Chenost found that the fibrous index (1/10 watt x hour) of the 25 hays studied exhibited a close relationship with the digestibility and acceptability of the hays, determined by animal experiments. Organic matter digestibility was more highly correlated with the fibrousness index ($r = -.931$) than with crude fiber ($r = -.756$). It would appear that repeatability of results would be impossible unless exactly the same equipment and method of feeding the samples into the pulverizing equipment are employed.

5. In vitro rumen fermentation analysis

A number of excellent reviews on the history and development of *in vitro* rumen fermentation techniques has been published (Moxon and Bentley 1955; Bentley 1959; Johnson and Dehority 1963; Johnson 1966). Many of the researchers working with *in vitro* rumen fermentation techniques have attempted to apply these techniques to evaluating the nutritive value of forage. A number of criteria used to evaluate the nutritive value of forage has been predicted from *in vitro* measurements.

a. Digestible energy criteria

(i) Digestible dry matter (DDM)

The use of *in vitro* fermentation for estimating forage digestibility and intake has been reviewed by Barnes (1965). In this review Barnes first explains that *in vitro* rumen fermentation techniques involve three ingredients including (1) the forage substrate, (2) an "artificial saliva," more properly a buffer and nutrient solution, and (3) the rumen inoculum. This mixture is incubated under anerobic conditions at 39°C. for a specific period.

Clark and Mott (1960) studied 11 forages of known *in vivo* digestibility, and found *in vitro* digestibility estimates obtained during the spring to be significantly correlated ($r = 0.77$) with the *in vivo* data. However, when the *in vitro* trials were repeated in the fall, a reduction in digestibility occurred and the correlation coefficient was reduced to 0.49. They postulated that the reduction in dry matter digestibility may have been due to decreased activity of the rumen microflora or changes in the stored forages. Bowden and Church (1962) also found discrepancies in the *in vitro* and *in vivo* relationship using forage samples stored for different lengths of time.

The work of Tilley *et al.* (1960) illustrates the effect of protein content and a proteolytic enzyme on the *in vitro*, *in vivo* dry matter digestibility relationships. These workers found the closest agreement between the *in vivo* and *in vitro* dry matter digestibility with feeds of low digestibilities and low-protein content. Feeds with higher digestibilities and particularly those with high protein contents revealed greatest discrepancies. Further work revealed that a secondary digestion with the proteolytic enzyme, pepsin, resulted in higher correlations ($r = 0.98$) compared to the correlation coefficient of 0.90 for rumen fluid alone. The use of pepsin simulated the digestion of protein which occurs post-ruminal.

Further research by Tilley and Terry (1963), using 130 samples of grass and 18 samples of clover and alfalfa, enabled them to establish the prediction equation $Y = 0.99X - 1.01$ with a standard error of the estimate of 2.31, where Y = digestible dry matter and X = *in vitro* digestible dry matter. This procedure utilized a 48-hour incubation time and an additional 48-hour incubation with pepsin, so therefore was quite time-consuming.

A highly significant *in vitro*-*in vivo* correlation (r = 0.996) for digestible dry matter was also obtained by Wedin *et al*. (1966) using the Tilley and Terry *in vitro* rumen fermentation procedure. These workers suggest that this method is relatively inexpensive and could be adapted where forage evaluation programs are underway. However, the time required to handle one sample greatly restricts the number of forage samples that can be handled during a given time.

Preliminary experiments were conducted by Karn *et al*. (1967) to determine at which *in vitro* fermentation time, measurements should be made to estimate maximum digestion rate. Both cellulose digestion (CD) and digestible dry matter (DDM) *in vitro* exhibited the highest correlation with *in vivo* DDM after an 11-hour digestion period. Rate of *in vitro* CD and DDM between 5 and 11 hours was similarly correlated with *in vivo* DDM with the correlation coefficients of the same magnitude as the 11-hour digestion data. None of their correlation coefficients was as high as those observed by Wedin *et al*. (1966).

Recent work by Dehority *et al*. (1968) suggests that *in vivo* DDM of forages can be estimated by cellulose digestion by pure cultures of cellulolytic rumen bacteria.

This new approach utilized seven strains of rumen bacteria and revealed that results from four of the seven strains of the cellulolytic bacteria were highly correlated with *in vivo* DDM.

Previous to the work of Kumeno *et al*. (1967) *in vitro* fermentation experiments had used a cellulosic material (forage or purified cellulose) as the substrate to be evaluated. Kumeno and co-workers studied and developed an *in vitro* fermentation technique for estimating the nutritive value of digestibility of high-energy mixed rations. Twelve mixed rations, consisting of various proportions of hay and concentrate (ground corn) were compared with digestion trials conducted *in vivo* (sheep) and *in vitro*. These workers observed that dry matter disappearance *in vitro* after 48 hours was highly correlated ($r = 0.85$) with *in vivo* digestible dry matter. The application of *in vitro* fermentation techniques to high-energy rations would appear to be logical in present-day feed evaluation programs.

(ii) Digestible energy (DE)

A comparison has been made by Hershberger *et al*. (1959) between *in vitro* cellulose digestibility and *in vivo* DE. These workers utilized inoculum prepared from sheep micro-organisms and a 24-hr. fermentation period for their *in vitro* work, along with conventional 11-day *in vivo* digestion trials. The forages tested were orchardgrass, bromegrass, Kentucky bluegrass, timothy, alfalfa and ladino clover. Cellulose digestion *in vitro* was observed to be significantly correlated (r = 0.92) with *in vivo* DE and a prediction equation was developed to calculate DE from per cent cellulose digested *in vitro*.

Baumgardt *et al*. (1962) used a simplified artificial rumen procedure to determine the relationship between *in vitro* cellulose digested and *in vivo* DE. These workers observed a high correlation between DE and *in vitro* cellulose digestion (r = 0.85) warranting the development of a prediction equation.

Digestible energy *in vivo* was not highly correlated with *in vitro* cellulose digestibility by seven pure strains of cellulolytic rumen bacteria according to the work of Dehority *et al*. (1968). However, when *in vitro* cellulose digestibility was multiplied by per cent dry matter solubility (1 N H SO), a high correlation (r = 0.90) was obtained with digestible energy.

(iii) Total digestible nutrients (TDN)

Pigden and Bell (1955) were among the first to use artificial rumen *in vitro* produce to evaluate forage quality. These workers combined *in vitro* methods for determining digestible organic matter and carbohydrate to predict TDN.

A simplified artificial rumen procedure was utilized by Baumgardt *et al*. (1962) to predict TDN. Per cent TDN could best be predicted from *in vitro* cellulose digestion according to these workers.

b. Nutritive Value Index (digestible energy intake potential)

Nutritive Value Indices (NVI) as developed by Crampton *et al*. (1960) were first determined by *in vitro* methods by Donefer *et al*. (1960). Nine forages, harvested over a period of two years and representing five different species (3 legume, 2 grasses) cut at various stages of maturity, chopped, and artificially dehydrated were studied by *in vivo* and *in vitro* tests. Donefer and co-workers determined Relative Intake and energy digestibility by *in vivo* (sheep) trials and calculated NVI of each forage. The 12-hour *in vitro* cellulose digestion determination was found to be highly correlated

($r = 0.91$) with NVI. These workers proposed that Nutritive Value Index Y could be predicted from 12-hour *in vitro* cellulose digestion (X) by the equation, $Y = -7.8 + 1.314X$.

Two years later, work by Johnson *et al.* (1962) confirmed the validity of the prediction equation proposed by Donefer *et al.* (1960). However, in their work Johnson and associates observed that the correlation coefficient of 0.95 between NVI and 12-hour *in vitro* cellulose digestibility (IVCD) of grasses, dropped to 0.86 when alfalfa hays were included. The validity of the 12-hour IVCD to predict NVI was substantiated by Johnson *et al.* (1965).

Methods of estimating forage nutrient value from *in vitro* cellulose digestion (IVCD) were studied by Chalupa and Lee (1966). These studies indicated that intake (Voluntary Intake, Relative Intake) and Nutritive Value Index (NVI) could best be estimated from 18-, 30-, and 18 x 30-hour, IVCD values, respectively. Chalupa and Lee suggested NVI could best be predicted from 18-hour IVCD by the prediction equation: $NVI = 25.5 + 1.20X$, where X is IVCD.

The Nutritive Value Indices (NVI) of forages fed ground or chopped using an *in vitro* fermentation method were determined by Donefer *et al.* (1962). Twelve-hour *in vitro* cellulose digestion coefficients (Donefer *et al.* 1960) were determined for samples of dry forage for which NVI had been obtained by feeding the forage in the chopped and/or ground form to sheep. The NVI of 26 forages fed chopped and 16 forages fed ground were highly correlated with *in vitro* cellulose digestion of the respective forages. Prediction equation to calculate NVI from 12-hour *in vitro* cellulose digestibility revealed that grinding of the forage increased NVI an average of 10.9 units over chopped forage.

Another approach in the determination of NVI was the use of chemical methods to dissolve cellulose. Dehority and Johnson (1963) used cupriethylene diamine (CED) to solubilize the cellulose of grasses. These workers were able to refine their technique to the extent that CED cellulose solubility was highly correlated with NVI. Dehority and Johnson proposed that NVI could be predicted by the equation: $NVI = 1.126X - 30.27$, where X = CED cellulose solubility expressed in per cent.

A slightly different approach for determining Nutritive Value Index (NVI) was taken by Donefer *et al.*

(1963). These workers used per cent dry matter disappearance (DMD) by enzyme and aqueous solution as the criteria to predict NVI. For 14 forages DMD as determined by HCl solution + pepsin was found to have the highest correlation (r = 0.95) with NVI determined *in vivo* with sheep. The correlation relationship between DMD by HCl + pepsin and NVI was considerably higher than the relationships observed by Dehority and Johnson (1963).

Donefer *et al.* (1966) reported on the development of a prediction equation to predict the digestible energy intake potential (NVI) from per cent dry-matter disappearance (DMD). In this experiment 49 forage (hay) samples, including both grass and legumes from five widely different locations were used to determine the relationship between *in vivo* NVI and DMD by pepsin-HCl solution. The *in vivo* data used was calculated from sheep and cattle. Digestible energy intake potential (NVI) was highly correlated (r = 0.95) with DMD for all forages.

6. Relative Intake (RI)

A number of researchers have attempted to establish highly significant correlations between RI and *in vitro* fermentation techniques. In most instances these correlations have not been high enough to establish reliable prediction equations. However, when chemical solubility of cellulose was combined with the *in vitro* criteria the relationship was improved. Johnson *et al.* (1965) observed that RI would only be predicted accurately from *in vitro* cellulose digestion when it was combined with dry matter solubility (1 N H_2SO_4).

Relative Intake (RI) relationships were determined by Dehority *et al.* (1968) using pure cultures of cellulolytic rumen bacteria. When the RI of eight grasses and four alfalfa were separately correlated with *in vitro* cellulose digestion by two strains of cellulolytic bacteria significant correlations were observed. However, when the two species groups were combined the correlation relationship was greatly reduced. Dehority and co-workers were not able to obtain a significant correlation between the two strains of cellulolytic bacteria and RI until RI was multiplied by (1 N H_2SO_4) dry matter solubility.

Donefer *et al.* (1966) observed a highly significant correlation (r = 0.94) between RI and pepsin - HCl dry matter disappearance for 49 forages. This correlation relationship was higher for all forages than for either grasses or legumes. The high correlation reported by

Donefer and associates indicates RI could be accurately predicted from pepsin - HCl dry matter disappearance. As RI is incorporated into the calculation of the Nutritive Value Index (NVI) it might not be necessary to separately predict forage intake, but rather use the more complete NVI measure.

The whole field of _in vitro_ laboratory methods for estimating forage quality was reviewed by Barnes (1965). He points out that the development of reliable laboratory methods for estimating forage quality is one of the most challenging problems in agriculture today. He emphasizes that the main criteria for the _in vitro_ analyses are dry matter and cellulose disappearance, although gas production and volatile fatty acid production are also utilized. The combination of _in vitro_ fermentation and enzymatic breakdown by pepsin has shown considerable promise. However, Barnes indicated that the precision or reproducibility of the _in vitro_ method is one of its greatest problems, and a standard forage is often employed in an attempt to measure some of the variability. The deserved accuracy of predicting _in vivo_ results from _in vitro_ data are standard errors of the estimate of not greater than two for digestibility and not greater than five for Nutritive Value Index.

The magnitude of *in vitro* digestion and the variability associated with the *in vitro* rumen fermentation technique was investigated in a collaborative study involving 17 laboratories by Barnes (1967). Considerable variability was observed in the techniques employed by the different laboratories. After observing the various sources of variability, Barnes concluded that the development of a standard *in vitro* procedure appears to be necessary if a more direct comparison of *in vitro* results among laboratories is desired.

D. Laboratory methods for evaluating corn silage

Coppock and Stone (1965) suggested the relative ease with which good-quality corn silage can be consistently harvested and stored without extensive losses probably accounts for the small amount of research done with corn silage in recent years, in contrast to the many experiments reported with grass-legume silages. However, as corn silage has become more widely accepted, researchers have started to look for methods of determining the nutritive value of corn silage. In general the trend has been to apply methods used to evaluate hay and grass silage.

1. Proximate analysis

The "proximate principles" have been investigated as predictors of nutritive value criteria (e.g., TDN, digestible dry matter, digestible energy) of corn silage in much the same way as with hay and grass silage.

a. Digestible energy criteria

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Nevens et al. (1954) investigated the relative proportion of leaf and ear of corn plants, suitable for silage during five successive seasons. Ear dry matter (DM) was expressed as a percentage of total DM in the silage and increased rapidly over short periods (viz., from 11.4 to 48.4% in 25 days. These workers observed a high positive correlation (r = 0.96) between ear DM and total DM, enabling the prediction of ear content of corn silage in which there was little change in the DM. The remaining DM in the leaf stalk remained almost constant.

German workers, Nehring and Laube (1958) utilized digestibility trials with sheep and chemical analysis to evaluate corn silage at different stages of maturity. The DM content varied from 13.0 to 22.3%, with the following constituents expressed on a dry matter basis: organic matter 92.8 to 95.7%; crude protein 7.1 to 14.2%; crude fiber 24.8 to 31.2%; nitrogen-free extracts 47.7 to 62.3%. These workers reported digestion coefficients of organic matter were 68.0 to 75.5%, and digestible protein 3.6 to 9.8%. With maturity, content of nitrogen-free extract was observed to increase significantly while both crude protein and crude fiber decreased.

A decrease in crude protein and cellulose of corn silage was observed by Johnson and McClure (1968). Since cellulose is indicative of the fibrous portion of plants these findings confirm the work of Nehring and Laube (1958).

After a five-year study of forage evaluation technique as applied to corn and grass silages, Week and Yegian (1965) concluded that the moisture content of silages seems to be the most important criterion of silage quality and hence feed value can be easily related to crop production and management practices. They observed that as moisture content of corn silage decreased from 82 to 70%, dry matter losses from seepage decreased from 10.0 to 0.5%.

Johnson et al. (1966b) determined the effects of corn plant maturity on changes in dry matter and protein distribution. Corn plants were harvested at six stages of maturity in 1962 and eight stages in 1964. Johnson and associates observed changes in dry matter from July 20 to October 14 were from 14 to 36% for stalks, 19 to 79% for leaves and 10 to 62% for ears. The highest total dry matter yield per acre appeared to be between the dent and glaze stages. These workers did not observe any vegetative growth of leaves or stalks during visible ear growth and maturation. However, Johnson et al. (1966a) reported that soluble carbohydrates of both stem and leaf increased for 5 weeks and then declined. In both years the ear constituted over 60% of the dry matter at maturity but did not reach this level until September 12 1962 and October 6 1964.

The effects of corn plant maturity on digestibility of corn silage in sheep was investigated by Johnson and McClure (1968). Thewe workers observed that the dry matter content of corn silage increased steadily from milk stage to fixed maturity. The apparent coefficients of digestibility were affected only slightly by maturity and were still 68% at the mature stage. Organic matter digestibility followed a similar trend ranging from 69.5% to 72.9% over all stages of maturity.

Cornell researchers (Coppoch and Slack 1966) have used the results of Johnson and McClure to provide an easy estimation of dry matter digestibility (DDM). When per cent DDM was plotted against per cent moisture or dry matter content of corn silage a linear relationship was obtained. By extrapolation of this line a table was produced to enable the estimation of per cent DDM at any moisture content.

Adams <u>et al</u>. (1964) published a formula for calculating TDN. This formula was of the same type as the other formulas for calculating TDN for other forages, whereby TDN was calculated from crude protein and crude fiber, both on a dry matter basis. These formulas are all prediction equations, which Adams and associates state were based on data from over 700 forages taken from world literature.

b. Voluntary intake and Nutritive Value Index

The importance of voluntary intake was demonstrated by Huber et al. (1963). Their work showed that the voluntary intake of milking cows (expressed as lb. dry matter/100 lb. body weight) increased from 1.95 to 2.13 to 2.31 as the silage dry matter increased from 25.3 to 30.3 to 33.2%, respectively. Since the TDN content of these three corn silages were essentially the same, TDN (digestible energy) intake increased as the dry matter of the silage increased. The relatively high voluntary intake by sheep of the more mature corn silage was observed in a two-year trial by Johnson and McClure (1968).

McCullough (1962) developed a multiple regression equation to predict silage dry matter intake (DMI) for dairy cows. In this prediction equation DMI was predicted from crude protein crude fiber and calculated TDN requirement for milk production. These four factors accounted for 93% variation in average silage DM content. The factors for the prediction equation were obtained from an experiment where 34 silages (viz., summer grasses, alfalfa, small grass and corn and silages made with and without additives) were fed free-choice and supplemented to dairy cows.

Another prediction equation was developed by McCullough (1962) from the data in the above experiment. Nutritive Value Indices (NVI) were best predicted from per cent crude fiber, dry matter and dry matter loss in the silo. This prediction equation was not as accurate as the one to predict dry matter intake because only 73% of the variation of NVI could be accounted for by the factors used.

The validity of voluntary intake as a measure of the nutritive value of silages was investigated by McCullough *et al.* (1965). In this experiment 59 silages of oats, lucerne, wheat, millet, sorghum and corn were studied in different years with lactating cows or grown heifers ranging in liveweight from 200 to 600 lb. Concentrates were fed to all cows at the same rate per unit milk produced. McCullough and associates were able to show that 87% of the variation in milk production could be accounted for by intake of digestible dry matter and differences in body weight.

2. <u>Other chemical analyses</u>

There has been very little work reported in the literature on the application of chemical analysis, other than the "proximate principles" to determine the nutritive value of corn silage.

a. Digestible energy criteria

Digestible dry matter by sheep was correlated by Simkins and Baumgardt (1963) with different chemical analyses to determine if reliable prediction equations could be developed. In this work they used acid detergent fiber (ADF), acid detergent lignin by Van Soest and digestible laboratory nutrients (DLN) by Thurman and Wehunt. The silages used in the experiments reported by Simkins and Baumgardt included corn silage as well as silages from many other species. However, the results of the work indicated that of the chemical methods tested, only acid detergent lignin was significantly correlated with DDM but this was only at the 5% level.

3. *In vitro* rumen fermentation

a. Digestible energy criteria

Attempts by researchers to predict digestible energy criteria from *in vitro* methods have met with little success. Simkins and Baumgardt (1963) attempted to predict *in vivo* digestible dry matter content (DDM) of corn and sorghum silage from *in vitro* cellulose and dry matter digestion. Their work revealed that DDM could not be accurately predicted for either *in vitro* cellulose digestion or *in vitro* dry matter digestion.

The cellulose digestibility of different parts of the corn plant was investigated by Johnson *et al*. (1966a). *In vitro* digestibility after 12- and 48-hr. of the cellulose of stem declined for 15 days and then remained constant. Johnson and associates observed that *in vitro* cellulose digestibility of leaf material was higher but declined slowly and steadily throughout. *In vitro* digestibility of cellulose was high for silage as for fresh material. However, these workers did not attempt to correlate *in vitro* cellulose digestion with *in vivo* criteria of nutritive value.

III. OBJECT OF RESEARCH

Various laboratory methods have been proposed to evaluate the nutritive value of forages. These methods have generally been developed by comparison with the known nutritive value of pure stands of forage.

The object of the research herein reported was: (1) to study the nutritive value of forage produced in the province of Quebec using various laboratory methods and coding systems as the criteria; (2) to compare the efficacy of various laboratory methods for the evaluation of farm-produced forages.

IV. FORAGE SURVEY

A. Introduction

A survey of the nutritive value of farm-produced forage was initiated in August 1966 to include hay and silages harvested and prepared during that summer. Forage samples and descriptive information were collected on farms enrolled on the Dairy Herd Analysis Service (D.H.A.S.) program of Macdonald College. These farms were selected because they were being regularly visited by college personnel (D.H.A.S. supervisors) with detailed information potentially available on the nature of the dairy operation. The D.H.A.S. program was just getting established during this period, with 125 herds already enrolled on the program.

The D.H.A.S. is a dairy cow production testing program, providing milk analysis, cost analysis and grain feeding recommendations for each cow on the program. The D.H.A.S. utilizes a digital computer (IBM 1620) in its calculations and production of farm reports.

As approximately 75% of the dairy farmers enrolled on D.H.A.S. were French speaking, and the writer is not bilingual, it was necessary to rely on the D.H.A.S. supervisors to act as interpreters and to assist in gathering forage samples and descriptive information relative to the samples.

B. Experimental

1. Area and farms surveyed

The dairy farms surveyed were restricted to those on the D.H.A.S. program with an attempt made to obtain forage samples for all 125 farms enrolled on D.H.A.S. at that time (August-November 1966). However, due to problems of fitting sampling time in with the D.H.A.S. supervisors' daily routine, it was not possible to visit all herds. A total of 85 farms was visited and 269 hay, 75 corn silage, and 26 grass silage samples were obtained. Of the hay samples, 243 were "first cut" and 26 were obtained from "second cut" (aftermath) growth. Hay, silage (corn or grass) were collected on each farm, if available. In some cases only hay samples were available, no silage being prepared on the farm. In certain cases, hay sampling was done before corn silage preparation so that the supervisors collected silage samples on a subsequent visit to the farm.

The 85 farms visited were for the most part in southwestern Quebec. This was the area within approximately a 60-mile radius of Macdonald College and covering parts of 20 counties.

The D.H.A.S. farms surveyed could be characterized as commercial milk operations. Generally the farmers with purebred dairy herds enroll on the Record of Performance (R.O.P.) program and not in the D.H.A.S. program because in the latter the production records are not considered official by the breed associations. Dairy farmers with smaller herds (less than 20 cows) and lower incomes from milk are not generally enrolled on the D.H.A.S. program.

2. Sampling equipment

Hay samples were obtained from baled hay by using a sampling device constructed by the Department of Agricultural Engineering of Macdonald College (Figure 1). This sampling device was modelled after the Penn. State Forage Sampler. The tube of the sampler was made from a 50-cm. length of zinc-coated, 16-gauge electrical conduit pipe with a 2.5-cm. inside diameter. A flame-hardened cutting tip was soldered to the end of the sampler tube.

Fig. 1. L to R, electric drill with closure devise; sampler tube with cutting tip at bottom; rod to extrude sample core; hay sample in polyethylene bag.

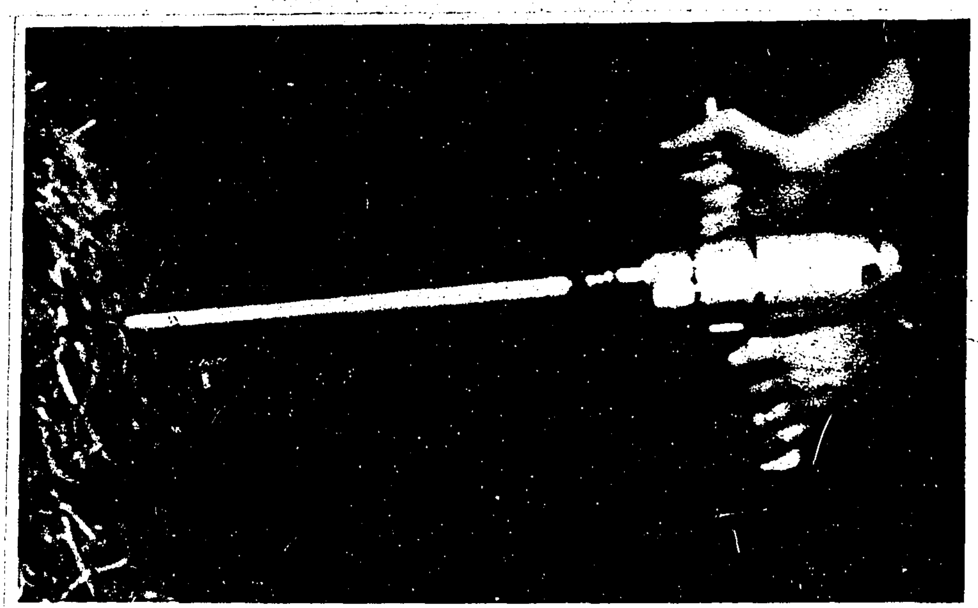
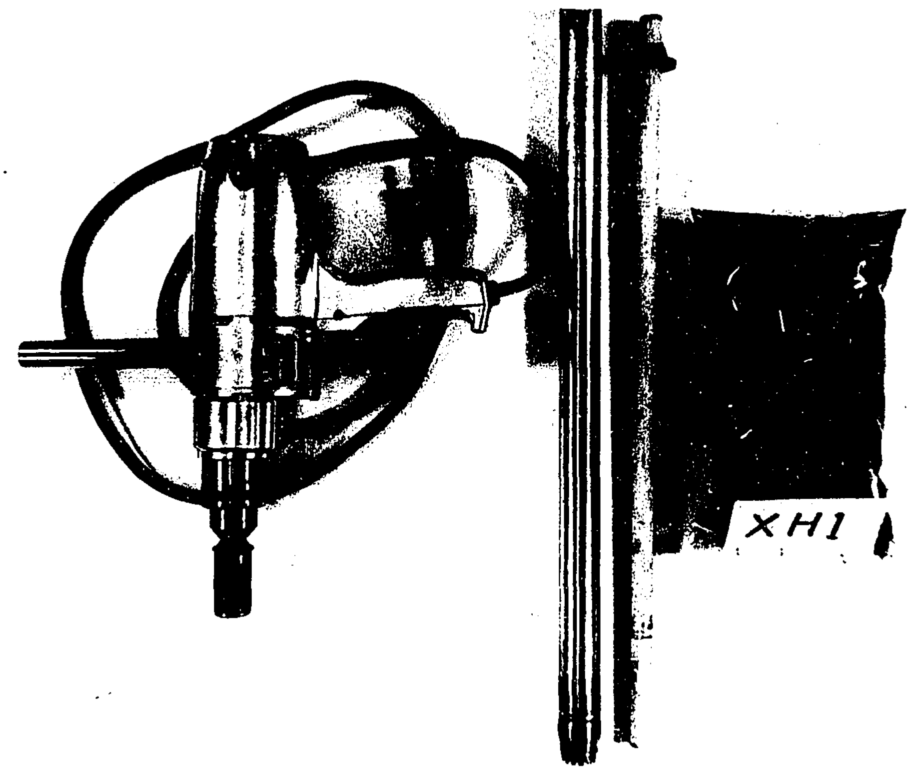


Fig. 2. Sampling procedure (hay).



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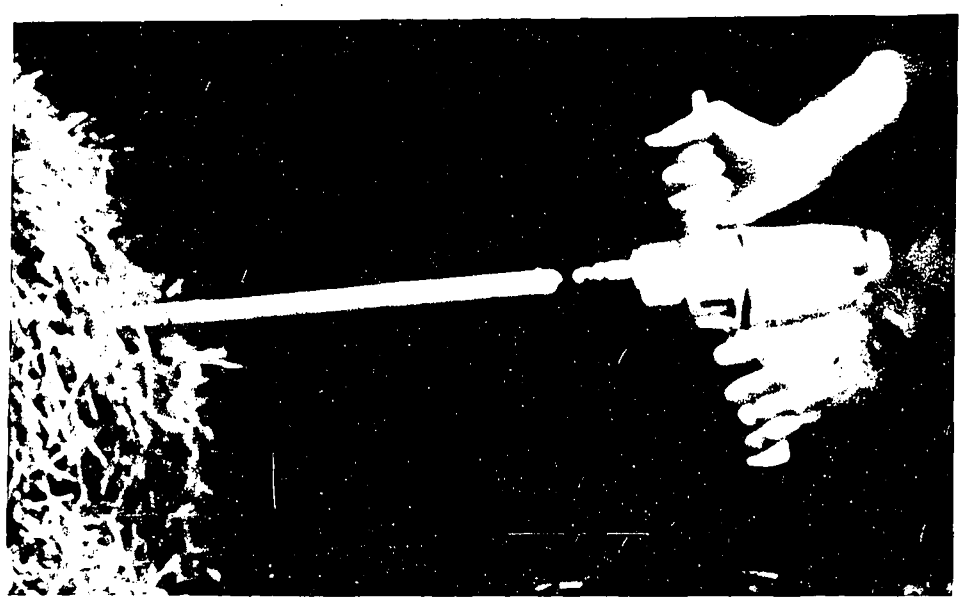

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The opposite end of the sampling tube was fitted with a closure device so that rotation of the tube detached it for sample removal. The closure end was soldered to a 1.0-cm. diameter rod which was inserted into the chuck of an electric drill (Wen model 950, 1/2 horsepower) to provide power for boring into the bales of hay.

3. Sampling procedure

a. Hay samples

Hay samples were collected by the writer accompanying the D.H.A.S. supervisor on his regular visits to participating farms. Forage sampling was undertaken after obtaining the permission of the farmer. For each type of hay, sample cores were randomly taken from 10-12 different bales located throughout the hay mow (Figure 2). These sample cores were taken by drilling into the exposed end of the bale. When loose hay was encountered, the hay was compressed by standing on it and sample cores taken by drilling vertically down into the hay. Where it was impossible to reach the hay in the mow, then bales of hay were thrown down to the barn floor and samples were taken.

Sampler emptying was accomplished by disconnecting the sampler at the closure device, placing this end into

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Sampler emptying was accomplished by disconnecting the sampler at the closure device, placing this end into a polyethylene bag (0.004 mm. thick) and pushing the hay core out of the sampler with a metal rod, approximately 62 cm. long and small enough to slide easily through the sampler. The polyethylene sample bags were sealed by folding the top over several times and stapling across the fold and an identification card listing the number of the sample.

b. Corn silage samples

Corn silage samples were collected either by drilling or taking several handfuls from the exposed surface of the silage. Any spoiled silage was removed before sampling. Cores or handfuls were taken from approximately six spots picked at random on the exposed face of the silo. The silage samples were contained in the same type of polyethylene bags as the hay samples. Care was taken to seal the bags tightly by more folds and securing with extra staples. These precautions were made to minimize loss of moisture from the samples.

The major proportion of silage samples were collected by the D.H.A.S. supervisors as it was not possible for the writer to visit farms after the first of October 1966. Through the courtesy of the D.H.A.S. supervisors, samples of silage were collected throughout the winter months of 1966-67 as the silage was fed out.

4. Information collection procedure (codes)

The third step in the sampling procedure was recording information describing the sample. A modification of the form used by D.H.A.S. and shown in Figure 3 was used.

Hay samples were numbered by the two letters "XH" followed by a number. The letter "X" was used to indicate the year 1966 while the letter "H" indicated a hay sample. The samples were numbered consecutively from number one to the total number of samples collected. Silage samples were numbered by the same method except the letter "S" was used to indicate silage.

Descriptive information relating to each sample was recorded in the appropriate column. This information was recorded by using the following codes.

a. Kind code (hay and grass silage)

This was used to describe the relative proportions of leguminous and grass species present in the forage. Forages were visually examined and an estimate made to the nearest one-third of legume and grass species present,

FORAGE HARVESTING RECORD

prepared by

Dairy Herd Analysis Service - Macdonald College, P. Que.

Member John Doe Herd Code: Co. 69 No. 201

H A Y

Sample number	Total yield (ton)	Kind of hay	Location in mow	Date harvested	Method of curing	Weather damage
XH1	100	1	west side	15-6-66	X	0

G R A S S S I L A G E

Sample number	Total yield (ton)	Kind of silage	Location in silo	Date harvested	Preservative used	
					Kind	Amt./ton
XS1	50	2	Top ½	1-6-66	-	-

Sample number	Total yield (ton)	Variety	Location in silo	Stage of maturity	Estimated bu. shelled corn/ton
XS2	50	Dekalb 30	bottom ½	2	-

Fig. 3. Form used for recording sampling information.

as follows:

Mixtures	Kind Code Number
Legume hay, 2/3 or more legumes	1
Mixed hay, 1/3 to 2/3 legumes	2
Grass hay, less than 1/3 legumes	3

b. Stage of maturity code
(hay and grass silage)

The stage of maturity of the plants as harvested was visually estimated according to the following codes:

Stage of Maturity	Maturity Code Number
Bud	1
Early bloom	2
Mid bloom	3
Full bloom	4
After bloom	5

c. Stage of maturity code
(corn silage)

Corn silage maturity as harvested was visually estimated and described according to the following codes:

Stage of Maturity	Maturity Code Number
Milk	1
Dough	2
Hard dent	3
Ripe	4

d. Method-of-curing codes
(hay)

The methods used to cure hay were coded as follows:

Method	Code Letter
Conditioned (stems crushed or crimped)	X
Mow dried (forced air)	Y
Artificially dried (forced air and heat)	Z
Sun cured	not coded

e. Weather-damage code
(hay)

Weather conditions during curing were described as follows:

Weather	Weather Code Number
No weather damage	0
Light rain	I
Heavy rain	II

In the column "date harvested" the actual harvest date was recorded. Columns headed "total yield" "location in the mow" "location in silo" and "variety" were completed for the farmer's and D.H.A.S. supervisor's benefit in planning the feeding program. The column headed "estimated bushels of shelled corn per ton" was not completed, while the column headed "preservative used" was rarely used due to infrequent use of grass silage preservatives.

The Forage Harvesting Record form (Figure 3) was completed in duplicate for each farm visited. One copy was left with the farmer and the second copy returned to Macdonald College with the forage samples.

C. Results and Discussion

1. Descriptive codes

a. Kind code

Distribution of first and second cut hays by kind code are summarized in Table 2. More than half of the first cut hays (58.4%) containing 2/3 or more grasses were coded as kind #3, while 80.8% of the second-cut hays containing 2/3 or more legumes were coded as kind #1. This data clearly indicates that legumes predominate in second-cut mixed stands.

When maturity code, weather code, and date of cutting (days after June 15) of first cut hays were compared according to species (kind), some interesting trends appeared as noted in Table 3. A trend was observed which indicated the higher the grass content

TABLE 2. Distribution of first-cut and second-cut hays by kind code

Forage	Kind Code*	First-cut hays No.	First-cut hays %	Second-cut hays No.	Second-cut hays %
Legumes	1	32	13.2	21	80.8
Mixed	2	69	28.4	4	15.4
Grasses	3	142	58.4	1	3.8
Total		243	100.0	26	100.0

* 1: 2/3 or more legumes
2: 1/3 to 2/3 legumes
3: less than 1/3 legumes

the more advanced the stage of maturity at harvest. Kind #3, with a mean maturity code of 4.5, was harvested at a significantly later stage of maturity than kinds #1 and #2, with mean maturity codes of 4.0 and 4.1, respectively.

This relationship between species and maturity was further confirmed in the date of cutting code (days cut after June 15) which increased with the increase in grass content. Kind code #3 was cut at a significantly later date than kind codes #1 and #2. The high standard deviation associated with the mean date of cutting code would indicate a large spread in harvest dates, especially

TABLE 3. Comparison of code data of first-cut hay according to species (kind) (243 samples)

Forage	Kind Code[1]	No.	Maturity code[2]		Date of cutting[3]		Weather code[4]	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
Legumes	1	32	4.0[a]	±0.4	19.4[a]	±15.3	0.4[a]	±0.9
Mixed	2	69	4.1[a]	±0.5	20.7[a]	±10.9	0.2[a]	±0.6
Grasses	3	142	4.5[b]	±0.5	24.6[b]	±12.9	0.3[a]	±0.8

[1]Kind code 1 = 2/3 or more legumes
Kind code 2 = 1/3 to 2/3 legumes
Kind code 3 = less than 1/3 legumes

[2]Maturity codes Nos. 1 to 5 indicate least mature to most mature

[3]Number of days after June 15 hay was cut

[4]Weather code 0 = no weather damage, while codes nos. 1 and 2 indicate light rain and heavy rain respectively.

[a,b]Means in the same column with similar superscripts are not significantly different ($P < .01$)

with legume hays which were harvested from approximately June 15 to the end of July.

No consistent relationship was found between weather and kind. However, there were not enough hays damaged by weather to be able to establish a relationship with any of the other factors considered.

b. Effect of cutting

Table 4 shows the comparison of first-cut hay, second-cut hay and all hays combined, according to kind, maturity and date codes. Second-cut hay contained significantly more leguminous plants than first-cut hay as indicated by a mean kind code of 1.2, and supports the data presented in Table 2. These data also indicate that second-cut hay was cut at a significantly earlier stage of maturity than first-cut hay.

Date of cutting code which is also an indicator of maturity does not follow the same pattern as maturity code, revealing no difference among hays. This difference may be due to second-cut hay being based on a different date. The base date for second-cut hays was the date that the earliest sample was harvested (August 1 1966) rather than June 15 1966, used for first-cut hay.

TABLE 4. Summary of code data for first-cut, second-cut and combined hays by visual appraisal

Forage	Kind Code[1]		Maturity Code[2]		Date of cutting[3]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
First-cut hay	2.5[a]	±0.7	4.3[a]	±0.6	22.9[a]	±12.9
Second-cut hay	1.2[b]	±0.5	3.3[b]	±0.9	23.8[a]	±10.2
Total	2.3[a]	±0.8	4.2[a]	±0.7	23.0[a]	±12.7

[1]Kind code 1 = 2/3 or more legumes
Kind code 2 = 1/3 to 2/3 legumes
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[2]Maturity codes 1 to 5 indicate least mature to most mature

[3]Days after June 15 hay was cut

[ab]Means in the same column with similar superscripts are not significantly different ($P < .01$)

Regardless of this, date code is an indicator of the mean number of days elapsing after the earliest harvested hay irrespective of the cutting of hay. However, date of cutting may have different effects for first- and second-cut hay as pointed out by Reid et al. (1959). These workers observed that digestible dry matter was highly correlated with days cut after April 30 until July 12. After July 12 the digestible dry matter decreased only slightly. This would indicate that it is quite possible that second-cut (aftermath) hay matures at a slower rate and is thus less mature at essentially the same "date of cutting" as first-cut hay.

V. A COMPARISON OF VARIOUS LABORATORY METHODS FOR EVALUATING FARM-PRODUCED FORAGE

A. Introduction

Different methods of evaluating forages were chosen for comparison. These methods were chosen because they appear to fulfill the criteria of a method for evaluating forage for use in practical feeding programs. These criteria were: (1) the method must be relatively simple so that the procedure can be carried out by regular laboratory personnel; (2) methods must be rapid so that large numbers of samples can be handled; (3) methods should not require expensive and overly complicated laboratory equipment; (4) the method must be able to measure the available energy content of the forages with sufficient accuracy to be utilized in practical feeding programs.

B. Experimental

1. Preparation of samples

All forage samples as described in Section IV were prepared for chemical analysis by being passed through the fine (0.59 mm. diameter) size screen (equivalent to 30 mesh U.S.B.S.) of a Raymond Laboratory Hammer Mill. Hay samples observed to be inadequately dried and all silage samples were dried overnight in a forced air oven at 45^{o}C. before grinding.

Each ground sample was thoroughly mixed and placed in an 8-oz., wide-mouth screw-top amber glass bottle. The bottle tops were fitted with a bakelite liner to make an air-tight seal. When full, these bottles contained approximately 75 gm. of sample.

2. Chemical analyses

All forage samples collected were analyzed for dry matter by the vacuum drying method of the A.O.A.C. (1965). Crude protein analysis was conducted according to the A.O.A.C. (1965) macro-kjeldahl method. The cellulose content of all samples was determined by the method of Crampton and Maynard (1938) as modified by Donefer _et al_. (1960).

3. <u>Additional chemical analyses</u>

Additional chemical analyses were completed on 40 selected hay samples. Due to the labor involved in the desired analyses it was impossible to do all analyses on the total (269) samples. Therefore, 40 samples were selected from the 269 samples for additional chemical analysis. The 40 samples provided a large enough number for error control in analysis of variance. These 40 samples were selected by a process of restricted randomization. The restriction was that there was a conscious attempt to select samples from all stages of maturity.

The 40 selected samples were analyzed for crude fiber according to A.O.A.C. (1965) with two modifications. First, a sintered glass pyrex filter base (Millipore no. XX 1004702) was used to replace the "Oklahoma filter." Second, liquid reagents were maintained at boiling by two hot plates instead of being immersed in a boiling water bath. Analyses for acid detergent fiber (ADF) and lignin were by the method of Van Soest (1963). Per cent cell contents was determined by the procedure developed by Van Soest and Wine (1967).

4. <u>Procedures to evaluate the energy content of forage</u>

a. Total Digestible Nutrients and Estimated Net Energy - The Penn. State Formulas

The methods used by Penn. State Forage Testing Service were utilized in the present study because these methods are currently being used in several states in the U.S.A.

(i) Total Digestible Nutrients (Penn. State - 1 formula)

The following formulas were used by the Pennsylvania State University Forage Testing Service (Adams 1961) until January 9, 1963 to predict TDN:

Digestible protein (DP) was predicted by the equation

$$DP = 0.946X - 3.52 \text{ (Holter and Reid 1959)}$$

where

X = crude protein as per cent of dry matter (DM)

Metabolizable energy (ME) as calories per kg. of DM was predicted by the equation

$$ME = 3240 - 14X_1 - 39.1X_2 \text{ (Axelsson 1952)}$$

where

X_1 = DP as per cent of DM

X_2 = crude fiber as per cent of DM

Total Digestible Nutrients (TDN) was calculated by the equation

$$\text{Per cent TDN} = \frac{\text{kcal. of ME per kg. DM}}{\text{3563 kcal. of ME per kg. TDN}} \times 100$$

where

3563 kcal. ME = 1 kg. TDN (Swift 1957)

For purposes of brevity, this method of calculating TDN will be referred to henceforth in this text as the "Penn. State - 1 formula."

(ii) Total Digestible Nutrients
(Penn. State - 3 formulas)

After January 11, 1963, the Penn. State Forage Testing Service changed from the 1-formula to 3-formulas to calculate TDN. The following prediction equations were developed by Adams *et al.* (1964) for different kinds of forage:

Legumes (including soybean forage and peavines)

$$\text{TDN} = 74.43 + 0.35\ \text{CP} - 0.73\text{CF}$$

Mixed hay (crops and forages of unknown origin)

$$\text{TDN} = 65.14 + 0.45\ \text{CP} - 0.38\ \text{CF}$$

Grasses and corn stover (no ears)

$$\text{TDN} = 50.41 + 1.04\text{CP} - 0.07\ \text{CF}$$

where

CP = per cent crude protein (DM basis) and

CF = per cent crude fiber (DM basis).

This method of calculating TDN will henceforth be referred to as "Penn. State - 3 formulas."

(iii) Estimated Net Energy (ENE)

TDN by either the Penn. State - 1 formula or Penn. State - 3 formulas, was converted to ENE by the formula by Moore et al. (1953). This formula is represented by the equation:

$$\text{ENE (megacal. per 45.4 kg.)} = 1.393X - 34.63$$

where

X = TDN as per cent of DM.

b. Total Digestible Nutrients and Estimated Net Energy - Van Soest Formulas

This method of determining the energy content was chosen for comparison because it was a new approach to evaluating forage and appeared to be a method that could be utilized in evaluating forage for practical feeding programs.

Van Soest and Wine (1967) method for calculating TDN utilized the following equations:

$$\text{Soluble cell contents (S)} = 100 - W$$

$$Y = 100L/ADF$$

$$R = 100\ Y/S$$

$$A = 100 - R$$

where

W = per cent cell wall constituents

Y = degree of lignification of fiber

L = per cent lignin

ADF = per cent acid detergent fiber

R = unavailable portion of forage and

A = availability index.

TDN can be calculated from the prediction equation

$$TDN = 0.653A + 16.7$$

where

TDN = lb. of total digestible nutrients per 100 lb. dry matter (or per cent).

ENE can be calculated from the prediction equation

$$ENE = 0.905A - 11.2$$

where

ENE = megacalories (Mcal.) per 45.4 kg. DM or therms per 100 lb.

Throughout the text the methods of calculating TDN and ENE by methods developed by Van Soest will be indicated as TDN (Van Soest) and ENE (Van Soest) respectively.

c. Nutritive Value Index (NVI)

Nutritive Value Indices were used as a measure of the nutritive value of forages in this study in an attempt to determine if NVI would be of practical value in a forage evaluation program. The concept of NVI was developed by Crampton *et al.* (1960).

NVI as developed by Crampton and associates was calculated from *in vivo* data by the following formulas:

$$\text{Relative Intake (RI)} = \frac{\text{observed daily hay intake (gm.)}}{80(W^{.75})} \times 100$$

where

W = weight in kg. and 80 gm. per unit metabolic weight is the daily consumption in gm. of the standard hay (good quality alfalfa) for sheep. For cattle the comparable figure is 140 gm. per day.

NVI = RI x % digestibility of energy

A laboratory method was developed by Donefer *et al.* (1966) whereby NVI could be predicted from dry matter disappearance (DMD) by incubation of a forage sample in an aqueous solution of pepsin-HCl.

NVI was calculated by the following prediction equation:

$$NVI = -0.75 + 1.60X$$

where

$$X = \text{per cent DMD}$$

As the above equation is based on hay samples on a dry-matter basis, which was not the case for the farm-produced samples, NVI was reported on an "as-fed basis" (predicted NVI x % DM of sample).

d. Evaluation of the energy content of hay by Quality Codes

The system of assigning Quality Codes to indicate the estimated net energy content of forages is being followed by a number of Dairy Herd Improvement Associations in the United States and is presently being utilized by the Macdonald College Dairy Herd Analysis Service. Due to the widespread application of forage Quality Codes the decision was made to compare this method of evaluating forages as developed by the Cornell University workers with the laboratory methods as described in 4a, b, and c of this chapter.

The Quality Code system used for evaluating hay was the Cornell Quality Codes as described in the DHIA Supervisors' Handbook (1962) as modified by the D.H.A.S. The cutting dates for the tabular code values were delayed one week to coincide with the later season of Quebec. The tabular values at various cutting dates can be observed in Table 5.

TABLE 5. D.H.A.S. Forage Evaluation Quality Codes

Date cut	Stage of growth	Quality codes
Before June 5	early vegetation	57
June 6 - 15	before heading or budding	50
June 16 - 25	boot stage or 1/10 bloom	43
June 26 - July 5	full bloom	37
July 6 - 20	after bloom	31
After July 20	ripe	25
Second and third cutting		41
Drop one grade if seriously damaged by weather		

Hay was coded by referring to Table 5 and finding the appropriate cutting date and stage of growth for the hay and assigning the tabular value indicated.

e. Evaluation of the energy content of corn silage

(i) By chemical analysis

TDN for the corn silage samples was calculated by the following prediction equation:

$$TDN = 77.07 - 0.75CP - 0.07\ CF$$ (Adams et al. 1964)

where

CP = per cent crude protein and

CF = per cent crude fiber, both on DM basis.

TDN was converted to ENE by the formula of Moore et al. (1953).

(ii) By Quality Codes

The system of assigning Quality Codes to indicate the estimated net energy content of forages also applies to corn silage. This system as developed by the Cornell workers gives estimated net energy value for different ranges of dry matter and stages of maturity. The tabular Cornell Codes were modified by D.H.A.S. for use in Quebec. These modified codes along with the original Cornell Quality Codes for the various dry matter ranges can be observed in Table 6.

TABLE 6. Corn Silage Quality Codes

Stage of maturity	% DM	D.H.A.S. Quality Code	Cornell Quality Code
Tasseled	22	48	58
Milk	24	49	59
Early dent	28	50	60
Hard dent	30	51	61
Ripe	35	52	62
Ears removed	23	-	-

f. Statistical analysis of data

Simple correlations were calculated for all parameters combination according to the method described by Steel and Torrie (1960). Multiple correlation analysis was carried out by the method described by Goulden (1952).

A randomized plot design was used to compare methods of determining ENE. Analysis of variance of this design was carried out as indicated by Cochrane and Cox (1957).

Treatment means were compared by Duncan's multiple range test (Steel and Torrie 1960).

Significant difference between two treatment means was determined by a "t" test described by Steel and Torrie (1960).

The IBM 1620 Digital Computer was utilized to execute the calculations of the statistical analysis as described above. The program for multiple correlation was written by Dr. John Moxley of the Macdonald College Computing Centre. Other data on D.H.A.S. herds were provided by computer programs written by Dr. Moxley. Operation of the computer, key punching and writing of assorted programs to handle data were provided by the author.

C. Results and Discussion (Hay)

1. Chemical Analysis

a. Crude fiber and cellulose (species relationships)

The Penn. State methods of estimating TDN and ENE were based on crude fiber and crude protein were utilized in the analysis of samples. Because of the time-consuming empirical aspects of the crude fiber analysis an alternative method of determining crude fiber was examined. The relationships of crude fiber and cellulose contents of selected samples of hay, grass silage, and corn silage are shown in Table 7. Means of crude fiber and cellulose for each type of forage are very similar. Standard deviations of crude fiber and cellulose analysis follow basically the same pattern, being small ($\pm$2.9 and $\pm$2.2) respectively, for hay while increasing in both grass silage and corn silage to approximately +5.0.

When crude fiber is compared as to type of forage there is not a significant difference between mean crude fiber content of hay and grass silage, but the corn silage is significantly lower than hay. However, crude fiber means of the two types of silages are not significantly different.

The percentage cellulose content of the forages follows exactly the same pattern as crude fiber.

Regression analysis was conducted between crude fiber and cellulose content of each type of forage with the relationships obtained illustrated in Figure 4. In the figure the similarity of the respective regression

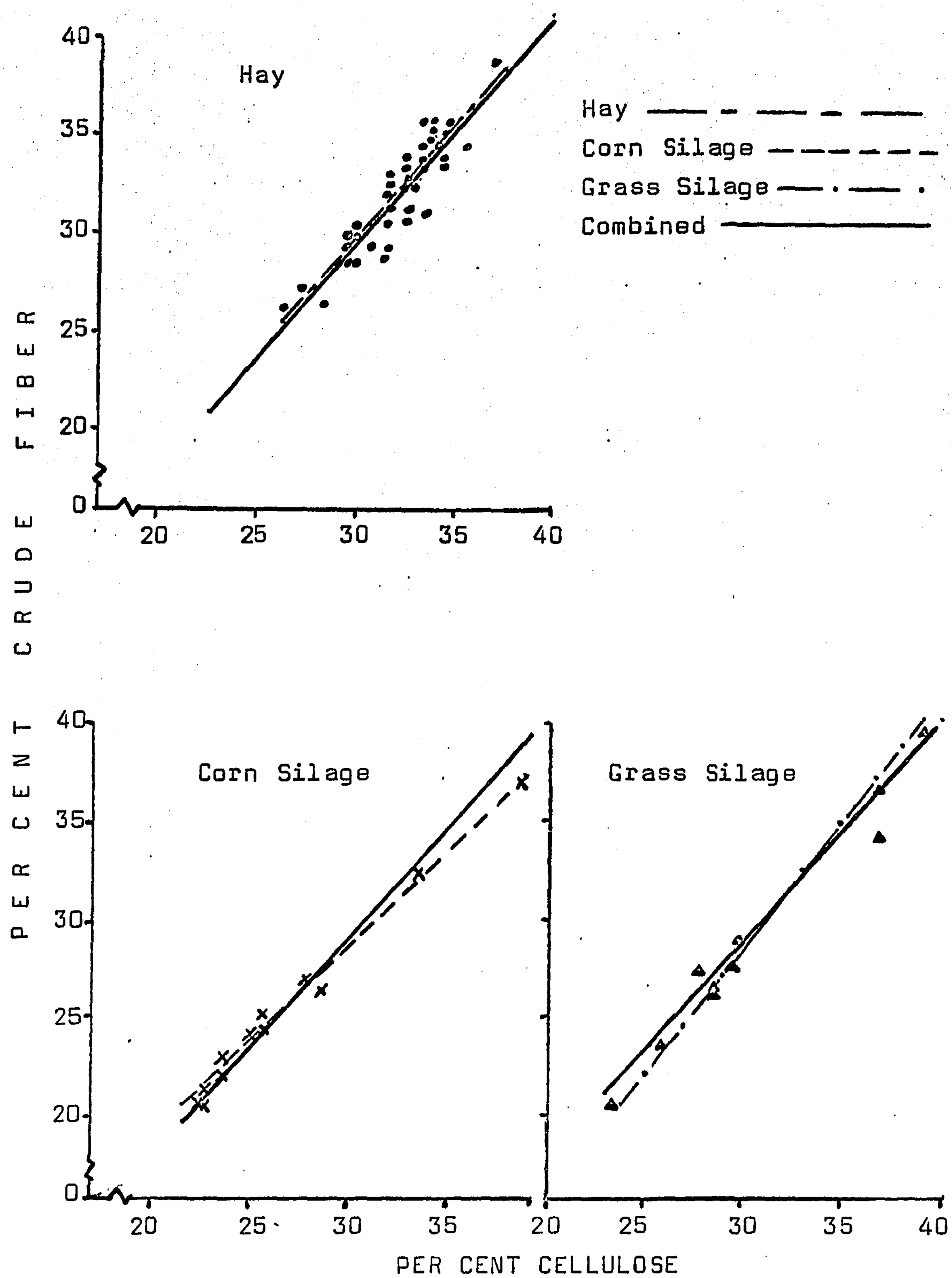

Fig. 4. Relationship between crude fiber and cellulose.

TABLE 7. Comparison of crude fiber and cellulose

Type of forage	Number of samples	Crude fiber Mean	Crude fiber S.D.	Cellulose Mean	Cellulose S.D.
Hay	40	31.9[a]	±2.9	32.1[a]	±2.2
Grass silage	10	29.2[ab]	±5.8	30.2[ab]	±4.3
Corn silage	12	25.4[b]	±4.9	26.7[b]	±5.0

[a, b]Means in the same column with similar superscripts are not significantly different ($P < .01$).

lines of crude fiber on cellulose for each type of forage and the combined types of forage can be observed.

In Table 8 the calculated regression equations, correlation coefficients, and standard error of the estimate are shown. When the standard error of estimating crude fiber is compared with the related crude fiber mean the per cent error is less than the standard 5% allowable variation between determinations. For example, for hay the calculation would be $1.21/31.9 \times 100 = 3.8\%$. The mean crude fiber figure was taken from Table 7.

Due to the inherent difficulties of the crude fiber analysis, plus the fact that cellulose determination is a routine analysis in our laboratory, it was decided

TABLE 8. Regression equations and reliability for estimating crude fiber (Y) from cellulose (X)

Type of forage	No. of samples	Regression equation	Correlation coefficient	Standard error of estimate
Hay	40	$Y=1.16X-5.28$	0.908**	1.21
Grass silage	10	$Y=1.33X-11.01$	0.982**	1.09
Corn silage	12	$Y=.99X-1.03$	0.995**	0.50
Combined	62	$Y=1.16X-5.39$	0.966**	1.19

**$p < .01$

to use the regression equations shown in Table 8 throughout this research project ot predict crude fiber from cellulose analysis.

The relationship between crude fiber and cellulose for grass silage and corn silage will be discussed later in Sections D and E, respectively.

b. Protein

The mean protein contents of first-cut and second-cut hays are presented in Table 9. Second-cut hay was significantly higher in protein than first-cut hay. This difference in protein content was due to a higher proportion of legumes in the second-cut hay as shown in Table 2, and

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TABLE 9. Summary of pertinent chemical analysis data for first-cut, second-cut, and combined hays on as-fed basis

Forage	Per cent Protein		Per cent Cellulose		NVI	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
First-cut hay	9.0[a]	±2.3	32.3[a]	±2.0	36[a]	±6.6
Second-cut hay	17.2[b]	±1.6	28.7[b]	±2.1	52[b]	±9.2
40 selected samples hay	10.9[c]	±3.5	31.7[a]	±2.5	39[ac]	±9.0
Total	9.8[c]	±3.3	32.0[a]	±2.3	38[c]	±8.3

[a,b,c]Means in the same column with similar superscripts are not significantly different ($P < .01$).

is a reflection of the generally higher protein content of legumes when compared with grasses. A rapid regrowth of legumes in unfertilized aftermath herbage was also observed by Mosi (1967).

The difference between protein of first and second cutting hay was in agreement with the observations of Colovos _et al_. (1949).

There was no difference in protein content of the 40 selected samples and the total samples. This was expected since the 40 selected samples were selected to be representative of the total samples.

Variation of crude protein due to kind of hay can be observed in Table 9. There was a concomitant significant decrease in protein as the kind changed from legume hay to grass hay. This data is in agreement with the many feed composition tables.

When variation of protein due to kind was compared for the 40 selected samples in Table 11 a similar pattern was observed, although the mean protein content was higher in each case.

c. Cellulose

Cellulose analysis (Table 9) showed that the mean cellulose content of second-cut hay was significantly lower than first-cut. Mosi (1967) and Woefel and Poulton (1960) observed a similar variation of cellulose. The standard deviation for cellulose reveals the relatively small, but consistent variability in cellulose content. Although only second-cut hay was significantly lower in cellulose content, there was an obvious inverse relationship between cellulose content and crude protein content of the four different groupings of hays.

When the effect of species on cellulose content was examined in Table 10 there was not a significant difference. Work by Van Soest (1967) and Colburn and Evans (1967) indicated a similar lack of relationship between cellulose and kind of hay.

Contrary to these results are the results of cellulose content in Table 11. The cellulose content of the 40 selected samples showed the mixed hay samples to have a mean cellulose content significantly greater than either the legume or the grass hay. Mean cellulose content of the legume and the grass hay was also significantly ($P < .05$) different. It is doubtful if these differences are meaningful, but show significance due to the random sampling of a small number of samples.

d. Nutritive Value Index (NVI)

The type of hay affects NVI by producing a significantly higher NVI for second-cut hay as indicated by Table 9. Mean NVI for first-cut hay and the 40 selected samples are the same due to the influence of the 32 first-cut hays in the 40 selected samples. However, when the NVI mean of the forty selected samples and total samples were compared there was not a significant difference, which was desirable, since the 40 selected samples were

TABLE 10. Comparison of chemical data of first-cut hays by kind (243 samples)

Forage	Kind code*	No.	Per cent Protein		Per cent Cellulose		NVI	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
Legumes	1	32	11.8[a]	±2.8	31.7[a]	±2.8	42[a]	±9.3
Mixed	2	69	9.8[b]	±1.9	32.5[a]	±1.6	38[b]	±5.5
Grasses	3	142	8.0[c]	±1.5	32.4[a]	±1.9	34[c]	±4.9

$P < .01$

*Kind code #1 = 2/3 or more legumes
#2 = 1/3 to 2/3 legumes
#3 = less than 1/3 legumes

[a,b,c]Means in the same column with similar superscripts are not significantly different

selected to be representative of the total number of samples. The high NVI of the second-cut hay was due to the high proportion of legumes in second-cut hay. The analysis data in Table 9 clearly picture the positive association of crude protein and NVI when compared by type of hay. On the other hand, cellulose exhibits a negative association with both crude protein and NVI.

In Table 10 NVI can be observed to decrease significantly as kind changes from legume to grass. This decrease is to be expected since workers at Macdonald College have amply demonstrated that grasses

TABLE 11. Comparison of chemical analysis of 40 selected samples

Forage	Kind code*	No.	Per cent Protein		Per cent Cellulose		NVI		Per cent ADF		Per cent lignin		% Cell Contents	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Legumes	1	8	16.3[a]	±1.7	29.2[a]	±1.8	53.0[a]	±6.3	36.0[a]	±3.0	7.4[a]	±0.9	51.8[a]	±5.3
Mixed	2	12	11.1[b]	±2.8	32.7[bc]	±1.4	37.6[b]	±5.7	40.2[b]	±1.5	6.8[a]	±0.8	39.6[b]	±6.2
Grass	3	20	8.6[c]	±1.3	32.0[ac]	±2.6	34.2[b]	±5.0	40.6[b]	±2.0	6.6[a]	±0.9	36.6[b]	±4.5

*Kind code #1 = 2/3 or more legumes
Kind code #2 = 1/3 to 2/3 legumes
Kind code #3 = less than 1/3 legumes

[a,b,c]Means in the same column with similar superscripts are not significantly different ($P < .01$)

have a lower NVI than legumes (Crampton et al. 1960; Donefer et al. 1960, 1963). This same relationship between kind code and NVI is evident in the 40 selected samples (Table 10).

e. Distribution of hay samples

After observing the differences between first-cut hay and second-cut hay with respect to crude protein, cellulose and Nutritive Value Indices (NVI), it was evident that there were two distinct populations. The distribution of the total hay samples (269) according to NVI, is presented by a histogram (Fig. 5). This histogram shows the per cent of the samples occurring in each range. The spread of all ranges is five, with the number shown being included in the range preceding it (i.e., the range with a mid-point of 25 includes 22.1 - 27.0).

The data graphically show that the distribution of 1966 samples by NVI fall into two distinct populations. Second-cut hay does not show a normal distribution and there is considerable overlapping of the two distributions.

Data were available from 104 samples of the 1967 hay crop from records of the Macdonald College Feed Testing Service and are compared with the 1966 data in

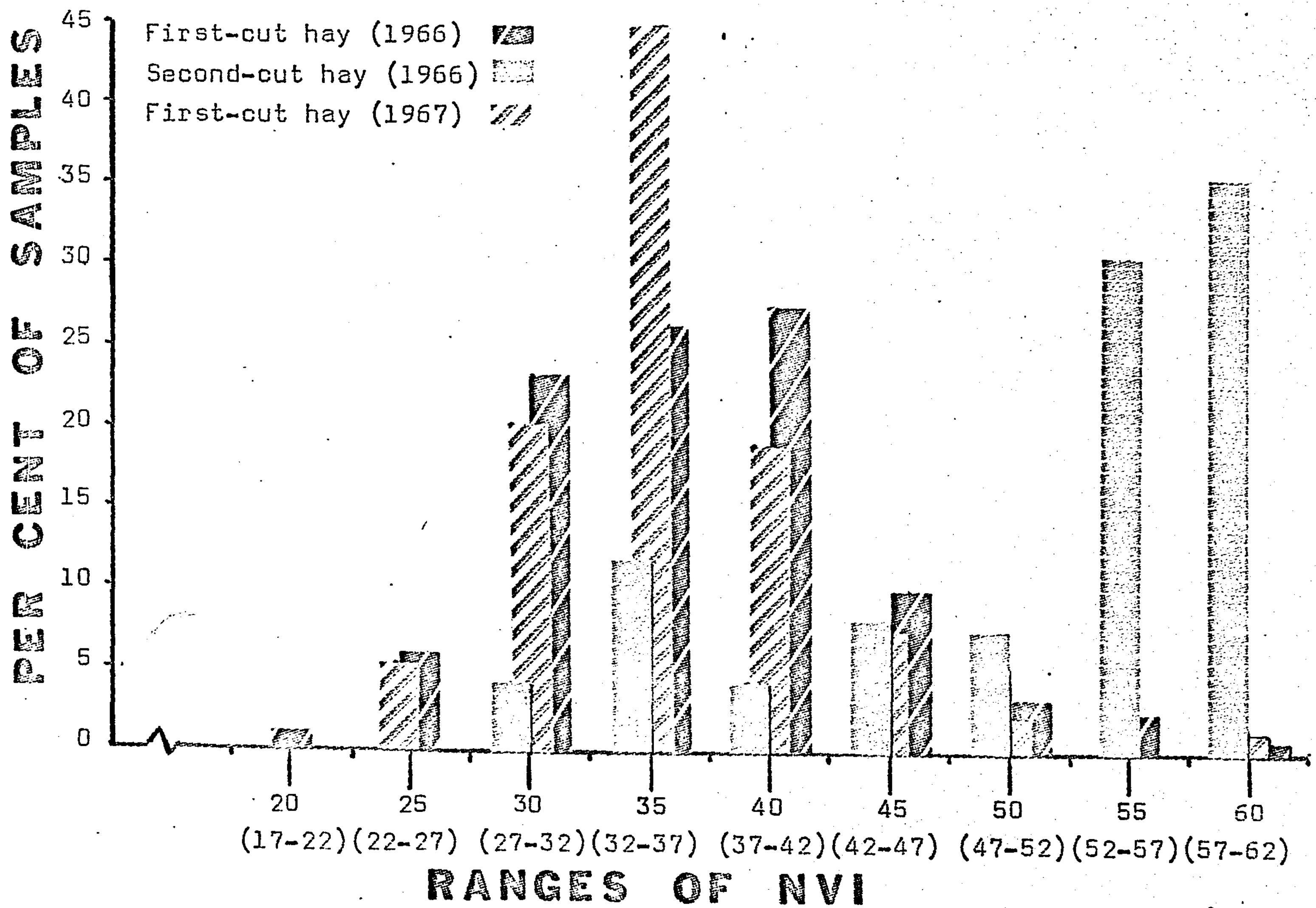

Fig. 5. Distribution of 269 hay samples by Nutritive Value Index.

Figure 5. The distributions of these data by NVI are basically similar although the 1967 data show a narrower distribution than 1966 NVI.

The distribution of 1966 and 1967 samples by crude protein are illustrated by Figure 6. Here the spread of each range is 2% (i.e., the range with a mid-point of 7% protein includes all samples with a crude protein content of 6.1 to 8.0%). As seen in Figure 6, crude protein content divides the samples into two separate populations according to cutting. These two populations have approximately normal distribution with the only overlap indicated in ranges with mid-points of 13 and 19% protein.

First-cut hay samples harvested in 1967 do not show as wide a variation as 1966 hays. This was indicated by a greater per cent of the samples occurring in ranges with mid-points of 9 and 11% protein.

There are two possible explanations of the difference between the distribution of 1966 and 1967 hay samples. First, the number of 1967 samples (104 first-cut) was less than half of the 1966 samples (243 first-cut) and would contribute to a narrower range of variation. The second and not so obvious reason for the sample

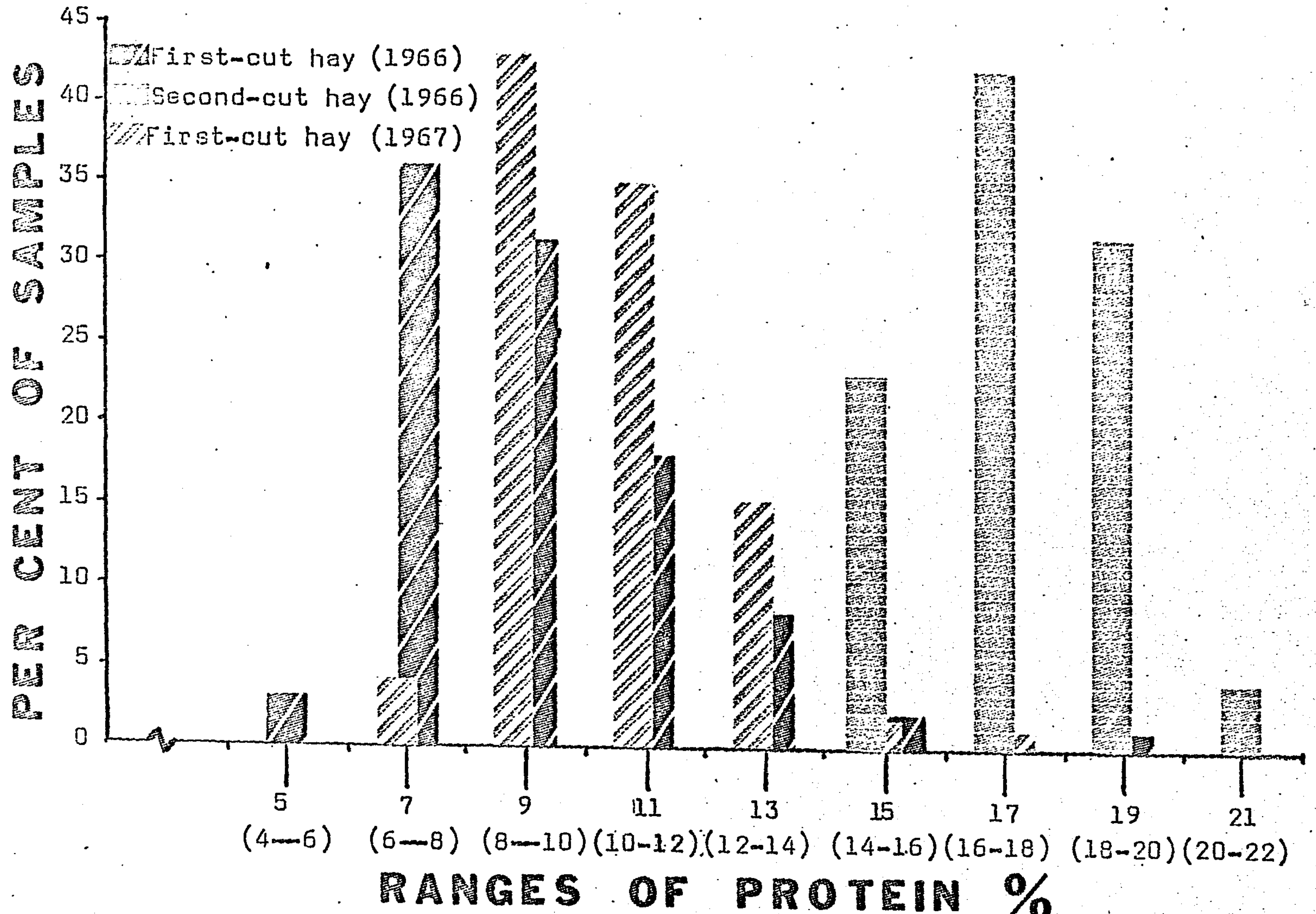

Fig. 6. Distribution of 269 hay samples by protein.

population difference was the method of collecting the samples. In 1966 a conscious effort was made to gather samples from all types of hay harvested as the samples were to be used as part of the research project with no charge to the farmer for the analysis. In 1967 as part of the Feed Testing Service a fee was charged and consequently farmers sending in samples for analysis would limit their samples to one or two of their main types of hay.

f. Acid detergent fiber (ADF)

Hay containing 2/3 or more than legumes (kind #1) was significantly lower in ADF than either groups containing less legumes and more grass (kind #2 or kind #3). Table 11 demonstrates this relationship and also indicates that mean ADF content of kinds #1 and #2 are not significantly different. The low ADF indicates a lower fibrous portion of legumes (kind #1) compared to grasses (kind #3) legume content of kind #1. This work is consistent with the work of Van Soest and Moore (1965) which indicated that ADF content of legumes is lower than ADF content of grasses. These findings were also in agreement with the work of Colburn and Evans (1967).

g. Lignin

Lignin is not affected by kind as indicated by Table 11 at the 99% probability level. However, at the 95% probability level, mean lignin content of kind #1 samples is significantly higher than kind #3.

The higher lignin content of kind #1 (2/3 or more legumes) can be explained from Van Soest's (1967) work which pointed out a special relationship between lignin and hemicellulose. Although legumes contain less hemicellulose, this hemicellulose is more highly lignified, which produces the low digestibility of hemicellulose from legumes when compared to the hemicellulose of grasses. Therefore, a higher lignin content of legumes would be expected. Crampton (1957) reported values for a small group of samples where the lignin content of legumes was 12% while grasses contained 8.5% lignin. Similar higher lignin content of legumes than grasses was reported by Colburn and Evans (1967).

h. Soluble cell contents

Soluble cell contents as determined by the method of Van Soest and Wine (1967) was significantly higher for legume hay, than either the mixed hay (kind #2) or grass hay (kind #3) as observed in Table 11. Mean cell contents of kind #2 tended to be higher than kind #3 but this difference was not significant.

These findings were in agreement with the work of Van Soest and Moore (1965), and Colburn and Evans (1967). These workers observed that generally cell contents were higher in legumes than in grasses but the results varied with individual samples. This would indicate that level of cell contents was influenced by other factors (e.g., maturity and weather).

i. Crude fiber

Crude fiber analysis was carried out on the 40 selected samples and the relationship between crude fiber and cellulose has been discussed previously in this text (p. 95). When crude fiber variation due to kind was examined, a relationship similar to cellulose was observed. Mean crude fiber content of kinds #1, #2, and #3 was 28.4, 32.7, and 32.8% respectively. The only observed difference was a slightly greater range in crude fiber content than for cellulose.

The results of Table 10 can be briefly summarized by the following observations: (1) lignin and cellulose showed very little variation with respect to kind of forage; (2) cell contents and NVI code were significantly higher, while ADF was significantly lower for mixtures high in legumes (kind #1); (3) crude protein showed the greatest within-kind variation, demonstrating significant decreases with increases in grass content (kinds #1, #2, and #3, respectively).

2. Simple correlations between all chemical and environmental parameters

a. Nutritive Value Index (NVI)

The correlations between chemical measures and coded environmental factors for first-cut and second-cut hays are compared in Tables 12 and 13.

Predicted NVI shows a highly significant correlation with protein content. In Table 14 the 40 selected samples and in Table 15 the total number of samples show similar significant correlations between NVI and protein. This relationship between NVI and protein was anticipated since work by Donefer *et al.* (1966) showed a significant correlation relationship with dry matter disappearance (DMD), and this soluble fraction would contain the soluble proteins. Crampton (1957) reported a positive correlation between crude protein and digestibility of forage, the latter being a component of NVI.

TABLE 12. Simple correlations between chemical and environmental parameters for first-cut hay (243 samples)

	Chemical analysis		Code data			
	Protein	Cellulose	Kind Code	Date Code	Maturity Code	Weather Code
NVI	0.518**	-.467**	-.456**	-.326**	-.275**	-.236**
Protein	--	-.084	-.587**	-.218**	-.408**	-.017
Cellulose		--	0.095	-.109	-.185**	0.067
Kind Code			--	0.163	0.326**	0.027
Date Code				--	0.631**	0.167
Maturity Code					--	0.112

** $p < .01$

TABLE 13. Simple correlations between chemical and environmental parameters for second-cut hay (26 samples)

	Chemical analysis		Code data			
	Protein	Cellulose	Kind Code	Date Code	Maturity Code	Weather Code
NVI	0.091	-.819**	-.194	0.252	0.068	-.369
Protein	--	-.077	0.213	0.038	0.006	0.073
Cellulose		--	0.012	-.401	-.132	0.304
Kind Code			--	0.191	0.409	-.060
Date Code				--	0.610**	0.096
Maturity Code					--	0.016

** P < .01

TABLE 14. Simple correlations between chemical and environmental parameters for hay (40 selected samples)

	Chemical analysis		Code data			
	Protein	Cellulose	Kind Code	Date Code	Maturity Code	Weather Code
NVI	0.792**	-.617**	-.746**	-.117	-.581**	-.112
Protein	--	-.445**	-.820**	-.277	-.732**	0.067
Cellulose		--	0.341	-.260	0.194	0.098
Kind Code			--	0.224	0.647**	0.097
Date Code				--	0.666**	0.197
Maturity Code					--	0.189

** P < .01

TABLE 15. Simple correlations between chemical and environmental parameters for all hay (269 samples

	Chemical analysis		Code data			
	Protein	Cellulose	Kind Code	Date Code	Maturity Code	Weather Code
NVI	0.673**	-.640**	-.569**	-.206**	-.437**	-.187**
Protein	--	0.400**	-.668**	-.122	-.561**	0.026
Cellulose		--	0.288**	-.127	0.087	0.062
Kind Code			--	0.135	0.472**	-.002
Date Code				--	0.536**	0.143
Maturity Code					--	0.065

**p .01

However, when only second-cut hay was considered there was essentially no association as measured by the correlation between NVI and protein (Table 13). This low correlation may be due to the high legume content of the second-cut hay with little variation in either protein content or NVI observed.

Significant negative correlations between NVI and cellulose were observed in Tables 12, 13, 14 and 15. In second-cut hay this correlation was higher than in the other hays and would seem to be due to the cellulose content of legumes showing more variability than the cellulose content of grasses. A negative correlation between cellulose and NVI is in agreement with the basic crude fiber concept as proposed by the Weende system of analysis, i.e., forage quality decreasing with increasing fiber content. Since cellulose is very closely related to crude fiber (Van Soest 1965) the same relationship was observed.

NVI and kind code revealed a significant negative correlation in all samples except second-cut hay (Table 13). This relationship which has been discussed in detail in the preceding section is confirmed by the highly significant correlation coefficients. The lack of this relationship with second-cut hay is a reflection of similar kind (legumes) and generally higher NVI values observed for these samples.

NVI was negatively correlated with date code in all hays except second-cut hay. The observed negative correlations were not as high as those reported between cutting days and digestible dry matter (DDM) by Reid _et al_. (1959) and Mellin et al. (1962). NVI is only partially related to DDM so correlation coefficients would not be expected to be the same between the literature and that herein presented. Cutting date from the work of Reid _et al_. (1959) was based on days after April 30, while Mellin _et al_. (1962) based their cutting date on days after May 17, and both these cutting dates were much earlier than the dates used in this work.

Second-cut hay (Table 13) again shows a different pattern from the other hays, indicating a low but positive association between NVI and date code. This difference may be due to a small number of samples, and some other variable (viz., kind) making the effect of date of cutting.

All hays with the exception of second-cut hays displayed significant but low negative correlations between NVI and maturity code. This relationship is in agreement with most research, which points out a loss of nutritive value due to advancing maturity (Davis *et al.* 1959; Jeffers 1960; Murdock *et al.* 1961). The effect of maturity may have been more pronounced if the population sampled contained hays harvested at earlier stages of maturity (Meyer *et al.* 1960). NVI and maturity codes of second-cut hay produced virtually no correlation and this was due to most of the second-cut hay having similar maturity codes.

NVI exhibited small, but significantly negative correlations with weather code in the first-cut hay samples (Table 12) and total hay samples (Table 15). In these two groups of hay the larger numbers permit numerically smaller correlation coefficients to show significance. The samples observed did not generally exhibit any degree of weathering so that the effect of this factor on forage nutritive value cannot be ascertained in this study. A negative effect of weathering on the nutritive value of hays, measured here by NVI, is generally accepted but may not be great, as indicated by Coetzee (1966), who compared digestibility of dry matter of lucerne hay. Coetzee (1966) found that neither prolonged periods of sunlight nor rain decreased digestibility.

b. Protein

Crude protein tended to decrease with a concomitant increase in cellulose content, particularly in the case of all samples (Table 15). This relationship was expected, since in the preceding section mean cellulose content of hay was influenced by species (kind) which influences protein level. The lack of high correlations between these factors is due to the lack of variability of cellulose content.

As indicated by the preceding section, protein and species (kind) have a close association in all cases except second-cut hay (Table 13). The correlation coefficient is negative because the legume hay which is the highest in protein has a kind code of #1. This relationship confirms published feedstuff composition tables, where legume hays contain more crude protein than grass hays. It is of interest to note that the highest correlation coefficient of Tables 12, 13, 14 and 15 was between protein and kind code. Again, second-cut hay shows a low correlation for this comparison and this is the result of most (80%) of the second-cut hay samples being the same kind (#1) and thus not varying greatly in protein content.

Crude protein content of hays tended to decrease as date code (cutting) increased as indicated by the negative correlation coefficients. The loss of protein due to later cutting dates was in agreement with the findings of Mellin _et al_. (1962) and Donker _et al_. (1968). This could be due to more leaf loss during the harvesting process. Reid _et al_. (1959) have established a high correlation between digestible dry matter (DDM) and leaf loss, although not the same as protein, there is an established relationship between protein and energy digestibility (Crampton 1957). As previously observed second-cut hay shows essentially no relationship between protein and date code. This lack of difference in second-cut hay is probably due to the protein content of the legume species not decreasing markedly with date of cutting.

Crude protein content and maturity code are negatively correlated for all hays with the exception of second-cut hay. These correlations are higher than the correlation of crude protein and date code which indicates that maturity code is a more accurate criteria for evaluating the effect of increasing stage of plant maturity on decreasing nutritive value. Since maturity code and date code are both determining plant maturity, the negative relationship between crude protein and maturity code was expected. The regression coefficient of crude protein on maturity code for the total samples reveals a drop of 2.8% crude protein per unit increase of maturity code. In the second cut hay (Table 13) the absence of any association between crude protein and maturity code was again prbbably a result of the relatively small decrease in nutritive value with maturity in regard to aftermath legumes.

Crude protein was essentially not affected by weather as recorded in this study. The absence of any real relationship between crude protein and weather code was in part due to an absence of any weather damage in the majority of samples.

C. Cellulose

Cellulose content shows relatively small effects due to species (kind code) with statistical significance appearing only with the total number of samples (Table 15). The low correlation coefficients indicate that the cellulose content of hay varies very little with kind of hay which is in agreement with Van Soest's (1967) conclusions regarding cellulose.

The negative correlations between cellulose and date code for all hays although statistically non-significant were opposite to expected results. It is suggested that the negative relationship is the result of the variation of cellulose content being relatively small between samples. The reason for a more pronounced negative correlation between cellulose and date code as observed in second-cut hay (Table 13) was maturity is more influenced by the date of cutting of first crop hay than the date which the second-cut hay was harvested.

Cellulose content of all groups of hays was inconsistently associated with maturity code. These correlation coefficients are a reliable indication of the random correlation which can occur when there is practically no variation of cellulose, and when the majority of hay samples were classified according to maturity codes as full bloom or after bloom (codes 4 and 5). The cellulose vs. maturity code correlation coefficient of first-cut hay indicates significance but only barely since the limit for $P < .01$ is 0.184.

Any relationship between cellulose and weather for all hays except second-cut hay are essentially non-existent. In second-cut hay the correlation coefficient between weather code and cellulose is high enough to suggest that rain may increase the cellulose content of hay, most probably as a result of decreases in soluble plant constituents.

d. Kind code

The small, but consistent, positive correlation between kind code and date code of all hays would indicate a trend toward grass hays being harvested at a later date than legume hays.

High positive correlations between kind code and maturity code for all groupings of hay verify the farmers' practice of cutting the more grassy hay (kind #3) at a later stage of maturity.

The correlations of kind code vs. weather code are meaningless and only serve to illustrate the low magnitude of correlation observed when there is no association between variables. In this way these correlations act as a control providing figures for variation due to chance.

e. Date code

Date code and maturity code are both measures of plant maturity so it is not surprising that Tables 12, 13, 14 and 15 record significant correlations of all groups of hay between these parameters.

The consistent positive correlation between date code and weather code would suggest that there was more rainy weather at the end of the 1966 haying season than at the beginning of the season. This suggestion is supported by the relationship of maturity code vs. weather code, which is quite natural since date code and maturity code are measuring the same effect. Since the preceding correlations are of a low order of magnitude they may be random effects without any practical significance.

f. "Van Soest" fractions

Similar correlations of cell contents, acid detergent fiber and lignin were calculated with all chemical analysis and code data for the 40 selected samples (page 85) and are presented in Table 16.

(i) Cell contents

The data in Table 16 show the simple correlations of cell contents, acid detergent fiber (ADF), and acid detergent lignin with chemical and code data. Cell contents and crude protein have a high correlation coefficient. This relationship is expected since protein

TABLE 16. Simple correlations of chemical analysis and code data vs. Van Soest analyses for 40 selected samples

Van Soest Analyses	Chemical analysis			Code data			
	Per cent Protein	Per cent Cellulose	NVI	Kind Code	Date Code	Maturity Code	Weather Code
Cell contents (%)	0.800**	-.694**	0.943**	-.704**	0.001	-.495**	-.083
ADF (%)	-.641	0.822**	-.830**	0.572**	-.060	0.405	0.019
Lignin (%)	0.367	-.064	0.228	-.332	0.188	-.132	0.015

** $p < .01$

is quite digestible and cell contents by Van Soest (1967) method represents the more digestible fraction of hay. In the analysis used to determine cell contents, the procedure separates the neutral detergent fiber and the remaining fraction is the cell contents which contains the available protein (Colburn and Evans 1967). Therefore, available protein content would certainly affect the per cent cell contents present in the hay.

When cell contents and cellulose were compared, a negative relationship was expected since cellulose is an indicator of the undigestible portion of hay. It is, however, surprising that the correlation coefficient is as high as indicated because sample cellulose content exhibited a small variability.

The highest simple correlation coefficient is observed when cell contents are compared with predicted NVI. This high correlation is a result of the similarity of methods involved in the determination for both cell contents and NVI. Cell contents by analysis are the soluble fraction of the cells comprising a particular forage, whereas NVI is predicted from dry matter disappearance dissolved by an aqueous solution of pepsin-HCl, thus also a measure of the soluble fraction of forage cells.

A high negative relationship between cell contents and kind code reveals progressive decreasing amounts of cell contents as the kind of hay changes from legumes to grasses.

Cell contents have no relationship with date code, moreover, in view of the indicated relationship between maturity code and date code, it can be concluded that date code is an inaccurate criterion for measuring maturity. The significant correlation between cell contents and maturity code underlines the fact that the digestible portion of hays is reduced with advancing maturity (Davis and Decker 1959; Mellin *et al*. 1962; Brown *et al*. 1968; Donker *et al*. 1968).

Per cent cell contents and weather code have no more than a random chance relationship. This relationship is a result of the majority of the hay samples having the same weather code (0).

(ii) Acid detergent fiber (ADF)

ADF and crude protein have a significant inverse correlation. Crude protein content is correlated with the digestibility of energy of forage (Crampton 1957) while ADF indicates the indigestible portion of forage, therefore the observed relationship is in agreement with expectations.

ADF and cellulose are both representative of the indigestible portion of forage, therefore a significant association would be logical.

NVI is observed to be highly correlated with cell contents; therefore the significant inverse relationship between ADF and NVI is also in keeping with expectations.

The relationship between kind code and cell contents has been previously discussed so it is not unexpected to find that the opposite relationship exists between ADF and kind code.

Date code as mentioned before, is not an accurate measurement of hay maturity as observed by the difference between correlation coefficients (ADF vs. date code and ADF vs. maturity code). The association between ADF and maturity code once again confirms the negative effect of maturity on the digestibility of hay.

(iii) Acid detergent (lignin)

Lignin and protein have a positive correlation due to the common influence of species, since legume hays generally contain more crude protein and lignin than grasses.

Relatively small standard deviations of lignin, cellulose, and weather code (as indicated in Tables 9 and 11) indicate that when these parameters are plotted, the points are clustered around the mean so closely that it is very difficult to establish any type of association between them and other variables.

Correlation coefficients between lignin and NVI and kind code are the result of the influence of species. As pointed out in the preceding section, legumes have a higher lignin content and NVI value than grasses.

The parameters, date code (date of cutting), and maturity code used as a measure of plant maturity are surprisingly only slightly related to lignin content. Highly lignified forages, such as straw, are not represented in the forages sampled.

(iii) Relationship of cell contents to lignin and acid detergent fiber (ADF)

In addition to the correlations reported in Table 16 for the 40 selected samples, additional correlation analyses were performed to determine the relationship between cell contents, ADF and lignin. Cell contents were found to be significantly correlated ($r = -.823$) with ADF. This was in accordance with the work of Van Soest (1967) which pointed out that ADF is an accurate index of indigestibility. Cell contents were correlated ($r = 0.332$) with lignin content of the 40 selected forages and this was due to the effect of species. ADF and lignin showed essentially no correlation ($r = 0.012$) and was due to the lack of variability of both ADF and lignin.

g. Multiple correlations

In order to determine the possibility of accumulative relationships between NVI and the other parameters of forage nutritive value, multiple correlation analyses were conducted with the results for first-cut hay samples presented in Table 17. The coefficient of determination (R^2) of NVI vs. all the parameters indicates that 57% of the variability in NVI is due to the parameters examined. Since this R^2 value indicates only slightly more than half of the variation in NVI for first-cut hay is accounted for, it is clear that there are additional factors not examined in this study, which might serve to

TABLE 17. Multiple correlation of Nutritive Value Index (y) vs. chemical composition and codes for first-cut hay

NVI (y) vs.	r	r^2 %	Partial Regression	Standard Partial Regression	R	R^2 %	S.E.[a]
Protein			0.910	.32			
Cellulose			-1.440	-.44			
Date Code			- .132	-.26	0.755**	57	4.3
Kind Code			-1.710	-.19			
Maturity Code			0.177	.02			
Weather Code			-1.254	-.16			
Protein			1.375	.48	0.671**	45	4.9
Cellulose			-1.404	-.43			
Protein	0.518**	31					5.6

** $P < .01$

[a]Standard error of the estimate of y

explain the observed differences in predicted NVI values.

The standard partial regression, which can vary from -1 to +1, was used to progressively eliminate the parameters contributing to the smallest per cent variation of NVI. The last two parameters, protein and cellulose, were responsible for 45% of the variation of NVI which demonstrates that the other parameters (date code, kind code, maturity code, weather code) account for only 12% of the variation. The major contributor to variation of NVI was protein, which accounted for 31% (r^2) of NVI variation (Table 13). By difference, the variation due to cellulose was 14%.

The multiple correlation relationships of NVI were also determined for second-cut hay (Table 18). NVI was correlated with the same parameters as were used for the first cut hay relationships, with 74% (R^2) of the variation of NVI accounted for by the six parameters examined. Cellulose and maturity code are responsible for 70% of NVI variability, while the coefficient of determination (r^2 from simple correlation, Table 14) indicates that 67% of this variation is due to cellulose.

Tables 17 and 18 show that the same parameters combined account for 74% of the variation of NVI for

TABLE 18. Multiple correlation of Nutritive Value Index (y) vs. chemical composition and codes for second-cut hay

NVI (y) vs.	r	r^2 %	Partial Regression	Standard Partial Regression	R	R^2 %	S.E.[a]
Protein			0.445	.08	0.860**	74	4.7
Cellulose			-3.298	-.77			
Date Code			0.026	.03			
Kind Code			-3.009	-.17			
Maturity Code			-1.348	-.12			
Weather Code			-1.356	-.15			
Cellulose			-3.497	-.81	0.837**	70	5.0
Kind Code			-3.294	-.18			
Cellulose	-.819**	67					5.3

**p < .01

[a]Standard error of the estimate of y

second-cut hay, but only 57% of the variation of first-cut hay. This discrepancy is due to the higher correlation between NVI and cellulose due to legumes in the case of second-cut hay ($r^2 = 67\%$) and has been discussed in the section dealing with simple correlations (Tables 13 and 14). In contrast, variation in NVI in first-cut hays was more accounted for by protein content ($r^2 = 31\%$) and less by cellulose content ($r^2 = 14\%$).

Multiple correlation relationships of NVI with the nine parameters investigated for the 40 selected samples are presented in Table 19. The high R^2 value (93%) and low standard error of the estimate (S.E.) value indicate that variation in NVI can almost be completely accounted for by the nine parameters examined.

When all the parameters, except soluble cell contents and ADF are eliminated, 90% of the variability of NVI was still accounted for. The coefficient of determination of predicted NVI vs. soluble cell contents indicates that 89% of the variation of NVI can be explained by measurement of soluble cell contents.

This relationship, although not able to account for quite as high a percentage of variation as the multiple correlation relationships, could have some practical

TABLE 19. Multiple correlation of Nutritive Value Index (y) vs. chemical analysis and codes for 40 selected samples

NVI (y) vs.	r	r^2 %	Partial Regression	Standard Partial Regression	R	R^2 %	S.E.[a]
Protein			-.340	-.13			
Cellulose			0.651	.18			
Soluble cell contents			0.951	.82			
ADF			-.937	-.28			
Lignin			-.261	-.03	0.966**	93	2.3
Kind code			-1.264	-.11			
Maturity Code			-1.112	-.11			
Weather Code			0.305	.03			
Date Code			-1.731	.02			
Soluble Cell contents			0.937	.80	0.949**	90	2.9
ADF			-.551	-.17			
Soluble cell contents	0.943**	89					3.0

$^{**}P < .01$

[a]Standard error of estimate of y

TABLE 19. Multiple correlation of Nutritive Value Index (y) vs. chemical analysis and codes for 40 selected samples

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Kind code			-1.264	-.11			
Maturity Code			-1.112	-.11			
Weather Code			0.305	.03			
Date Code			-1.731	.02			
Soluble Cell contents			0.937	.80	0.949**	90	2.9
ADF			-.551	-.17			
Soluble cell contents	0.943**	89					3.0

**P < .01

[a]Standard error of estimate of y

application, in that alternatively either dry matter disappearance (DMD) utilizing a pepsin-HCl solution or Van Soest's soluble cell contents measure could serve as laboratory predictors of forage NVI. The pepsin-HCl has the advantage of being a simpler and more direct method of analysis. In addition the validity of soluble cell contents as a predictor of NVI would have to be confirmed in studies with forage of known NVI (from *in vivo* trials) rather than the relationship with predicted values as reported in this study.

Four regression equations were developed from the simple and multiple correlation relationships. The equations developed and the parameters involved are presented in Table 20. Equation #1 could be used to calculate NVI from cell contents with an expected error of 7.7% (assuming relationship would be confirmed with forages of *in vivo* NVI values). This per cent error is higher than the allowable 5% but there may be times when a 7.7% error is permissible, viz., if other sources of error are equal or greater than this level. NVI can also be determined by equation #2, with slightly less error as measured by standard error of the estimate (S.E.), or per cent error of the predicted mean.

TABLE 20. Regression equations developed from simple and multiple correlations from 40 selected samples

Variables compared	Regression equation	No.	S.E.[1]	Per cent error of Mean (Y)
NVI (Y) from soluble cell contents (X)	$\hat{Y} = 1.10X - 5.60$	1	3.0	7.7
NVI (Y) from cell contents (X_1) and ADF (X_2)	$\hat{Y} = 22.72 + .94X_1 - .55X_2$	2	2.9	7.4
ADF (Y) from cell contents (X)	$\hat{Y} = .91X + 10.79$	3	1.6	4.0
Cell contents (Y) from DMD (X)	$\hat{Y} = 1.22X + 7.52$	4	2.3	5.7

[1]Standard error of estimating Y

Equation #3 provides a reliable method of calculating ADF with a small S.E. and per cent error of estimating ADF. This equation would eliminate the laboratory procedure of determining ADF, replacing it with the assay for cell contents. The relationship between cell contents and dry matter disappearance (DMD) (as determined by the pepsin-HCl method) by equation #4 provides a common link between Van Soest's work and the work of the Macdonald College workers. The small S.E. and 5.7% error of the estimated value of (Y) make equation #4 a reliable means of estimating cell contents from DMD.

3. <u>Calculated TDN and ENE values</u>

a. Means compared

Total Digestible Nutrients (TDN) were calculated by both Penn. State methods (pages 86 and 87) for all hays. Table 21 compares the two Penn. State methods of calculating TDN for the four groupings of hay samples.

The mean TDN content of the total of hay samples by "Penn. State - 1 formula" method was significantly different from similar means calculated by "Penn. State - 3 formulas" method. Significant differences were determined by a "t" test analysis for paired data.

TABLE 21. Summary of calculated TDN data for first-cut, second-cut, and combined hays on a DM-basis

Forage	Per cent TDN			
	Penn. State 1 formula		Penn. State 3 formulas	
	Mean**	S.D.	Mean**	S.D.
First-cut hay	57.7^a	±2.8	56.4^a	±2.0
Second-cut hay	65.7^b	±2.8	59.7^b	±2.8
40 selected combined	59.3^a	±4.0	57.5^a	±1.6
Total[1]	58.5^a	±3.6	56.8^a	±2.3

[1]Total of first-cut hay and second-cut hay

**Means of the two methods of calculating TDN are significantly different for each type of forage ($P < .01$)

[a,b]Means in the same column with similar superscripts are not significantly different ($P < .01$)

A comparison of TDN content of the four groups of hay by "t" test analysis for unequal groups revealed that second-cut hay was significantly different from the other three groups of hay. First-cut hay, 40 selected samples and the total samples were not found to be different. It is of interest to note that the 40 selected samples had the highest standard deviation (S.D.) and this was due to the number of second-cut hay samples in this group.

Calculation of TDN by "Penn. State - 3 formulas" revealed again that second-cut hay was significantly higher in TDN than the other three groups of hay, which were not significantly different.

Standard deviation of TDN calculated by "Penn. State - 3 formulas" method was lower except for second-cut hay than standard deviations when "Penn. State - 1 formula" was used. The low standard deviation reflects the effect of the weighting factors of the respective formulas for legumes, mixed hays and grass hays.

In Table 22 the TDN values by the two Penn. State methods have been converted to estimated net energy (ENE) by Moore's Formula (page 88). The same relationships exist among the four groups of hay for ENE as for TDN with one exception. The mean ENE content of "Penn. State - 1 formula" first-cut hay was significantly lower than the mean ENE of all other groupings. This difference is due to the influence of the second-cut hay (8 samples) in the 40 selected samples and (26 samples) in the total samples. There was no difference in the mean ENE between the 40 selected samples and the total number of samples as predicted by either of the Penn. State methods. Due to the effect of the constant of Moore's formula the standard deviation of all calculated ENE was consistently higher than the standard deviation of calculated TDN.

TABLE 22. Summary of calculated ENE data for first-cut, second-cut, and combined hays on a DM-basis

Forage	Per cent ENE (Moore's Formula)			
	1 formula		3 formulas	
	Mean**	S.D.	Mean**	S.D.
First-cut hay	45.8[a]	±3.8	44.0[a]	±2.8
Second-cut hay	56.9[b]	±3.9	48.6[b]	±3.9
40 selected combined	48.0[c]	±5.5	45.5[a]	±2.3
Total[1]	46.9[c]	±5.1	44.4[a]	±3.2

[1]Total of first-cut hay and second-cut hay

**All means calculated using 1 formula are significantly different from all similar means calculated by 3 formulas (P .01)

[a,b,c]Means in the same column with similar superscripts are not significantly different (P .01)

In summary of the data from Tables 21 and 22, it was evident that both TDN and ENE, regardless of the method calculated, was higher for the second-cut hays than any of the other groups. The TDN and ENE of the 40 selected samples and the total samples are the same irrespective of method calculated indicating that the 40 selected samples are representative of the total number of samples. It was evident that the two Penn. State methods tested do not give the same results, with "Penn. State - 1 formula" giving significantly higher TDN and

ENE values. This was due to the effect of the high-protein content of second-cut hay in the prediction equation.

In an attempt to compare various methods of determining ENE, a randomized design was utilized. Five methods of determining ENE were compared for the 40 selected samples since complete data for calculating ENE were only available for these samples. Nutritive Value Index (NVI), although not a recognized method of determining ENE, was included as one of the five methods compared. NVI was included in this comparison because it was shown to be highly correlated (r = 0.492) for Morrison's tabular ENE values which apply to the 40 selected samples. Also, it was one of the objectives of this study to compare NVI with other established methods of evaluating forage.

An analysis of variance established that there was a highly significant variation due to methods of determining ENE. This indicated that the methods utilized to determine ENE were not similar. Duncan's multiple range test (Steel and Torrie 1960) was used to determine differences between means. The results of this test are presented in Table 23.

TABLE 23. Means of ENE by five methods on an "as-fed" basis[1] (40 selected samples)

Method	Mean	S.D.
Penn. State - 1 formula	44.2[a]	±5.5
Penn. State - 3 formulas	41.9[ab]	±2.3
NVI	39.0[bc]	±9.0
Van Soest	36.5[c]	±5.8
Cornell Quality Code	35.4[c]	±5.4

[1]92.4% dry matter

[a,b,c]Means with the same superscript letter are not significantly different ($P < .01$).

Table 23 indicates that mean ENE as calculated by the "Penn. State - 1 formula" and "Penn State - 3 formulas" is not significantly different as indicated by Table 22. However, the comparison in Table 22 was made by a "t" test analysis for paired data which is generally considered more exact than Duncan's multiple range test. Mean ENE content by NVI, Van Soest and Cornell Quality Code was not significantly different. The ENE means of the 40 selected samples as indicated by NVI and "Penn. State - 3 formulas" were also similar.

In Table 23 the standard deviations (S.D.) of observations indicate that NVI varies over almost twice the range of the other methods of estimating ENE (viz., Cornell Quality Code, Van Soest, "Penn. State - 1 formula"). Mean ENE by "Penn. State - 3 formulas" shows the smallest S.D. of the five methods used to estimate ENE and this lack of variation among samples appears to be due to the weighting factors of the three prediction equations (page 87) used in this method.

b. Correlations compared

The five methods of determining ENE were compared with each other by simple correlation analysis. The resulting correlation coefficients are presented in Table 24. NVI is significantly correlated with three methods of determining ENE, viz., "Penn. State - 1 formula," Van Soest and Cornell Quality Code.

"Penn. State - 1 formula" is more highly correlated with NVI than "Penn. State - 3 formulas" because in the initial prediction equation to determine TDN (page 86) crude fiber or cellulose has a greater influence on TDN than in the "Penn. State - 3 formulas." Since NVI has a high correlation with cellulose, as shown in Table 13, then a higher correlation between NVI and "Penn. State - 1 formula" would be expected in the case where cellulose has the greater influence.

TABLE 24. Simple correlations of ENE methods from 40 samples

Method Compared	Penn. State -1 formula	Penn. State -3 formulas	Van Soest	Cornell Quality Codes
NVI	0.768**	0.379	0.584**	0.540**
Penn. Stete - 1 formula	--	0.578**	0.454**	0.266
Penn. State - 3 formulas		--	0.337	0.103
Van Soest			--	0.368

** P< .01

NVI is correlated with Cornell Quality Code due to a mutual relationship with digestible energy. Hardison (1959) pointed out that digestible energy (DE), digestible dry matter (DDM), and TDN are essentially the same, therefore Cornell Quality Code which is estimated net energy is directly related to DE and TDN by Moore's equation. NVI is also directly related to DE since NVI = Relative Intake x digestibility of energy.

"Penn. State - 1 formula" method of estimating ENE is significantly correlated with the "Penn. State - 3 formulas" method of estimating ENE and this relationship is due to the fact that both these methods utilize prediction equations involving protein and crude fiber. However, in the case of the "Penn. State - 1 formula" method (page 86), digestible protein is utilized rather than crude protein as in the "Penn. State - 3 formulas" method.

Penn. State - 1 formula" ENE is significantly correlated with Van Soest's ENE and this relationship is the result of the effect of the fibrous portions of forages being utilized in the respective prediction equations. Crude fiber is used in the "Penn. State - 1 formula" while the Van Soest method of determining

ENE (pages 87-88) uses acid detergent fiber and lignin.

"Penn. State - 1 formula" ENE is not significantly correlated with Cornell Quality Code. This low correlation is due to Cornell Quality Code being highly correlated with date code and kind code (Table 25), while showing very little relationship to protein or cellulose (crude fiber predicted from cellulose, page 99), which is utilized in the prediction of "Penn. State - 1 formula" ENE.

"Penn. State - 3 formulas" ENE is not significantly correlated with Van Soest ENE because crude fiber does not appear to contribute as much to the variation of "Penn. State - 3 formulas" ENE as does ADF and lignin to Van Soest ENE.

"Penn. State - 3 formulas" ENE shows very little association with Cornell Quality Code and is indicative of the lack of influence of crude fiber and crude protein on Cornell Quality Code (Table 25).

Van Soest ENE and Cornell Quality Code show a meaningful correlation which indicates that date code and kind code also influence the factors which contribute to the variation of Van Soest ENE.

TABLE 25. Comparison of coefficients of determination of chemical analysis and codes for 40 selected samples

Dependent variables	Independent variables	r^2 [b]	R^2 [b]	S.E. [a]
ENE				
Van Soest (y)	Protein (x_1) Cellulose (x_2) Kind Code (x_3) Maturity Code (x_4) Weather Code (x_5) Date Code (x_6)		35	4.7
$y.x_2x_6$			32	4.8
$y.x_2$		18		5.2
Cornell Quality Code (y)				
$y.x_1x_2x_3x_4x_5x_6$			81	2.4
$y.x_3x_6$			76	2.7
$y.x_6$		62		3.4
Nutritive Value Index (y)				
$y.x_1x_2x_3x_4x_5x_6$			76	4.5
$y.x_1x_2$			71	4.8
$y.x_1$		63		5.5

[a]Standard error of the estimate; [b]Coefficients of determination in per cent.

c. Coefficients of determination compared

After observing that several of the parameters were responsible for variation in all the five methods of determining ENE, an attempt to measure this variation was made by utilizing multiple correlation analysis. By means of multiple correlation, each method of estimating ENE was correlated with parameters which were known not to be involved in the actual calculation of a specific method of estimating ENE. For this reason the two Penn. State methods were not correlated with the common parameters because preliminary work indicated that practically all the variations could be accounted for by protein and cellulose.

In Table 25, three methods of determining ENE are correlated with six parameters to determine the variation accounted for by these independent variables. The six independent variables: protein, cellulose, kind code, maturity code, weather code and date code account for 35% of the variation of ENE (Van Soest). Two parameters, cellulose and date code are responsible for 32% of the ENE (Van Soest) variation. Approximately half the variation in ENE (Van Soest) is the result of the influence of cellulose, which would be expected, since cellulose is highly correlated with ADF (Table 14) and ADF is involved in calculating ENE by Van Soest's method.

When Cornell Quality Code was correlated with the same six parameters mentioned above, 81% of its variation was accounted for. Kind code (species) and date code accounted for 76% of Cornell Quality Code variation while date code alone had a coefficient of determination of 62%. Reid et al. (1959) showed that digestible dry matter (DDM) could be calculated from cutting days after April 30 and since DDM is positively correlated with ENE, it was not surprising to find a positive correlation between Cornell quality code and date code. It is of interest to observe a high association between Cornell Quality Code and date code when ENE (Van Soest) has relatively no association with date code. These observations would suggest that Cornell Quality Code is more closely related to digestible dry matter than is ENE (Van Soest).

Seventy-six per cent of the variation of NVI is accounted for by the six parameters being compared, however, the major portion of the variability can be traced to crude protein and cellulose content (71%).

Crude protein accounts for 63% of the NVI variation which is much more than the combined contribution of the other five parameters. This would be expected from the work of Crampton (1957) where crude protein was shown to be associated with voluntary consumption and digestibility of forage.

The standard error of the estimates (S.E.) are high enough as to indicate that the use of these regression equations would reduce accuracy, however, there may be situations where it would be practical to calculate Cornell Quality Code from kind code and date code. Presently Cornell Quality Code is assigned after visual appraisal using cutting date and stage of maturity as guides in this appraisal. In a case where appraisal of hay is being made by inexperienced personnel the following equation would be more accurate:

$Y = 48.01 - .30X_1 - 2.64X_2$

where

Y = Cornell Quality Code,

X_1 = date code, and

X_2 = kind code.

d. Variability of assigning
Cornell Quality Codes

The question of the accuracy of applying Cornell Codes by different individuals was examined, by having two D.H.A.S. supervisors and the author (R.F.W.) rate the 40 selected samples according to the Cornell codes. The assigned Cornell codes were compared with predicted NVI in Table 26.

There were no significant differences between the mean ENE of the 40 selected samples as assigned by the three individuals. No significant difference was observed between the coded ENE of the author and predicted NVI. The standard deviation observed for NVI was much greater than Cornell code standard deviation by the author, which in turn was greater than standard deviation of supervisor #1 or supervisor #2. The greater standard deviation of the author was believed to be due to following as closely as possible the suggested method for assigning Cornell codes (DHIA Supervisors' Handbook 1962), while the D.H.A.S. supervisors coded as they have been doing in the field. The relatively small standard deviation of Cornell codes by supervisors #1 and #2 indicates that these individuals code values close to the mean with very little variation.

TABLE 26. Comparison of the application of Cornell Codes and Nutritive Value Indices to 40 selected samples

Supervisor #1		Supervisor #2		Author (R.F.W.)		NVI	
Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
36.4^{ab}	± 3.3	35.8^{ab}	± 2.2	35.4^{a}	± 5.4	39.0^{b}	± 9.0

^{a,b}Means with similar superscripts are not significantly different ($P < .01$)

Correlation coefficients of Cornell codes and NVI were compared in Table 27. All correlation coefficients were positive and significant although there was a noticeably higher correlation between supervisors and the author than when supervisors were compared with NVI.

TABLE 27. Simple correlations of Cornell Quality Codes and NVI (40 selected samples)

	Cornell Quality Codes		
	Supervisor #1	Supervisor #2	Author (R.F.W.)
NVI	0.626**	0.599**	0.540**
Supervisor #1	-	0.750**	0.808**
Supervisor #2	-	-	0.741**

**p .01

e. Required accuracy of evaluating forage in practical feeding programs

In order to examine the accuracy necessary for evaluating hay under practical feeding conditions, a test herd was enrolled on the D.H.A.S. program. This test herd was a hypothetical herd producing varying amounts of milk and receiving different quantities of two types of hay. The object of this test herd was to utilize the computer to calculate the changes in recommended grain ration when the ENE value of the hay was changed by five units. The computer program was the same one used to calculate grain recommendations for actual herds enrolled in the D.H.A.S. program. Table 28 illustrates the possible changes in grain recommendation where the ENE is varied by 5 units at four different levels of hay feeding. Rate of roughage feeding is the amount of roughage fed kg. per 100 kg. of body weight. The feeding index is a measure of the per cent of total feed required supplied by hay. When hay consumption per cow was 9.0 kg. and the quality code (ENE) was increased by 5, the daily grain ration requirements were reduced by 16.4 kg. for 26 cows or 0.6 kg. per cow. Under practical farm conditions it is rather doubtful if the dairy farmer would achieve an accuracy of grain feeding of less than 0.6 kg. per day per cow. Therefore, under practical farm conditions, the quality code could be varied by as much as 5 units and not affect grain ration feeding if the level of hay consumption per cow is not above 9.0 Kg.

At higher levels of hay consumption the decrease in grain ration was more pronounced. When hay was fed

TABLE 28. Effect of varying hay quality code by 5 units on grain[1] requirements of test herd[2]

Hay consumption per cow			Rate of roughage feeding	Feeding index	Daily decrease of grain ration	
kg.	lb.	Code			per herd kg.	per cow kg.
9	20	35	1.5	32	0	0
9	20	40	1.8	36	16.4	0.6
11	25	35	1.9	40	0	0
11	25	40	2.2	45	20.0	0.8
14	30	35	2.3	48	0	0
14	30	40	2.7	54	27.7	1.1
16	35	30	2.3	48	0	0
16	35	35	2.7	56	30.5	1.2

[1]Grain ration calculated at 70% ENE

[2]Hypothetical herd composed of 26 Holstein cows with average production of 47.3 lb. of milk containing 3.6% fat

at the rate of 11.0 kg., 14.0 kg., and 16.0 kg. per day the recommended daily grain ration per cow was reduced by 0.8 Kg., 1.1 Kg., and 1.2 Kg., respectively, for an increment of 5 quality code units. These higher rates of hay feeding would necessitate more accuracy in evaluating hay. At the 16.0 kg. rate of hay feeding, an approximate variation of not more than ±3 quality code units would be desired. On the majority of the farms surveyed silage was being fed, in fact a survey of 28 D.H.A.S. herds revealed that an average of 9.0Kg. of hay was fed per cow during the months of February, March, April and May.

f. Application of NVI as an indicator of ENE

The decision was finally made to use NVI on a trial basis as an indicator of ENE of hay for the D.H.A.S. herds, as part of a Feed Testing Service offered by Macdonald College, starting September 1967. This decision was based on the following facts: (1) NVI is highly correlated with the four methods of determining ENE examined (Table 24); (2) Mean NVI of the 40 selected samples is not significantly different from mean ENE by Van Soest of the same samples (Table 23); (3) The mean Cornell Code of the 40 selected samples as established by two D.H.A.S. supervisors is the same as the mean NVI (Table 26); (4) NVI is significantly correlated with the Cornell Codes of all the three individual assigning codes (Table 26); (5) Under average practical dairy farming conditions, quality code or NVI values could be out 5 units and not adversely affect grain ration recommendations (Table 28).

The procedure followed when NVI was used to estimate ENE was to substitute laboratory determined NVI for the previously used Cornell Codes, as determined by visual appraisal. After the plan had been operational for approximately six months, an attempt was made to evaluate the results of using NVI as an indicator of ENE.

D.H.A.S. herds which had used NVI to indicate ENE for four months were selected. These herds were subdivided into three groups: (1) herds where ENE rating of hay was decreased 3 or more units; (2) herds where ENE of hay was increased by 3 or more units; (3) a control group where ENE was not changed. It was anticipated that if the hay was under-evaluated then the resultant increase in grain ration would be evident by more milk or greater body weight. In cases where hay may have been over-evaluated, the resultant decreases in grain ration might have produced lower milk production and/or loss of body weight.

Results of this comparative study are shown in Table 29. The parameters used to measure the variation of the three groups of herds revealed no differences except in the mean weight of cows in the herds where the

TABLE 29. Comparison of D.H.A.S. statistics of herd where Nutritive Value Indices were changed by 3 or more units

Treatment	Weight (kg.)		Feeding Index (%)		Herd Size (No.)		Production Index (kg.)		Hay Feeding (kg.)		% of ENE required from hay	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Code decreased[1]	472[a]	±63	102[a]	±12.2	34[a]	±6	13.9[a]	±3.6	8.5[a]	±2.5	43.0[a]	±19.5
Code increased[2]	526[b]	±23	110[a]	±7.9	35[a]	±13	14.5[a]	±3.3	9.0[a]	±4.5	49.1[a]	±27.3
Code not changed[3]	495[b]	±41	111[a]	±9.3	34[a]	±12	14.0[a]	±2.9	9.6[a]	±3.5	54.5[a]	±19.9
204 Herds[4]	512		104		34		14.8		5.5		27.5	

[1]Statistics based on monthly means of Feb.-May for 6 herds.

[2]Statistics based on monthly means of Feb.-May for 9 herds.

[3]Statistics based on monthly means of Feb.-May for 13 herds.

[4]Statistics based on monthly means for one year.

[ab]Means in the same column with similar superscripts are not significantly different ($p < .05$).

ENE code was decreased. This difference of body weight was due to several herds of the Channel Island breed being in this group. Production Index is average daily milk production of cows milking from 18 to 270 days. Feeding Index represents the ratio in per cent of total ENE supplied to the ENE required. Hay feeding indicates the number of kg. fed per cow per day, while the per cent of ENE required from hay represents the contribution of hay ENE to the total ENE required. Data on the 204 herds is the same as the other groups of herds for all parameters except "hay feeding" and per cent ENE from hay. These two parameters have much lower values due to the amount of ENE supplied from pasture during the summer months. It is interesting to note that the herds where ENE was increased had a "feeding index" of 110 which would compensate for any effects of underfeeding that were anticipated.

Two conclusions can be made from the study of the efficacy of NVI as an indicator of ENE. The first conclusion is that NVI is no worse and no better than the application of Cornell Code for determining ENE. A second and more valid conclusion is that with this type of field study there is just too much uncontrollable variation to permit any measurement of the effects of incorrect evaluation of hay. This study does substantiate the argument that under practical farm conditions, errors in hay evaluation have to be large before any effects will be observed.

D. Results and Discussion (Grass Silage)

1. Chemical analysis

The total number of grass silage samples suitable for analysis was twenty-four and hence information derived from studying these samples was not as reliable as information from the larger number of hay samples. There was not enough information available on kind (species) of grass silage, maturity and weathering to assign codes. However, these samples were analyzed for dry matter (dried in a forced air oven at 45°C.), crude protein, cellulose, and predicted NVI.

a. Dry matter

Mean dry matter content of the grass silage was 33.5% with a standard deviation of ± 13.1 as shown in Table 30. Since a dry matter content of 40-60% is generally considered haylage, the material sampled would have to be considered grass silage. The high standard

TABLE 30. Summary of pertinent chemical analysis data for grass silage (24 samples)

Sample form	Per cent Dry matter		Per cent Protein		Per cent Cellulose		NVI	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Silage: as fed	33.5	±13.1	4.8	±2.2	10.3	±1.5	12	±4.1
Silage: oven dried	91.6		14.3	±6.5	30.9	±4.4	36	±12.3
First-cut hay	92.4		9.0	±2.3	32.3	±2.0	36	±6.6
Second-cut hay	92.4		17.2	±1.6	28.7	±2.1	52	±9.2

deviation indicated that some farmers were allowing the grass to wilt before ensiling, while others were ensiling direct cut material. When the grass silage was dried overnight in the laboratory in a forced-air oven, the mean dry matter content was 91.6% which was similar to the dry matter content of first- and second-cut hay (92.4%). The protein and cellulose data for the hay samples are included in Table 30 for comparative basis.

b. Protein

On an "as-fed" basis grass silage contained 4.8% protein with a high standard deviation. The magnitude of the standard deviation can be compared with that of hay when silage protein was expressed on the oven dried basis (91.6% DM). The 14.3% protein content would indicate that the grass silage samples were comprised of a higher proportion of legumes than first-cut hay, but not as much as the second-cut hay samples. The high standard deviation indicates a much greater variability in protein content between different samples of grass silage than observed for the hays.

c. Cellulose

The high standard deviation of cellulose (dried basis) confirms the variability of grass silage, as indicated by protein analysis. This variability reveals the presence in the silage of both legumes and grasses. On the average the cellulose content of the dried silage was approximately half-way between that of first-cut hay and second-cut hay. Another explanation of the fact that grass silage was higher in protein (dried basis) than first-cut hay would be due to lower harvesting losses of protein-rich leaves in the case of the ensiled material. Culpin (1962) reported less losses of dry matter (leaves) for grass silage than for the same material stored as hay.

d. Nutritive Value Index (NVI)

Mean NVI content of the dried grass silage was exactly the same as that observed for first-cut hay samples. A high standard deviation of ± 12.3 is in agreement with the high standard deviation of protein and cellulose for dried grass silage, which points out again the greater variation in the plants ensiled from grass silage. The high NVI value (52) for second-cut hay would indicate that grass silage samples surveyed are definitely inferior on the basis of NVI values.

These data in Table 30 would suggest that it is the practice of farmers to ensile a wide variety of plants (grass and legumes). Quite possibly the farmer is motivated by the need to store forage under adverse weather conditions, rather than a desire to cut his forage at an earlier stage of maturity.

A comparison of the laboratory analysis of grass silage as provided by Table 30 would suggest that grass when dried has all the attributes of hay and hence a relationship established for hay in this text would also apply to grass silage, expressed on a dried basis.

E. Results and Discussion (Corn Silage)

1. Chemical analysis

a. Dry matter (dried basis)

Mean corn silage dry matter (DM) content was 26.6% with a standard deviation of ± 4.6 (Table 31). This would indicate that on the average, corn silage was not harvested at as high a dry matter content as grass silage (33.5% DM). The distribution of corn silage samples by per cent DM can be observed from Figure 7. Distribution of DM (dried basis) ranged from 17% to 41% with the highest percentage (40%) occurring within the range of 25% to 29%. This distribution of DM content

TABLE 31. Summary of chemical analysis and code data for 75 corn silage samples (as-fed basis)

Per cent Dry matter[1]		Per cent Cellulose		Per cent Protein		Maturity Code[2]	
Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
26.6	±4.6	6.6	±1.1	2.0	±0.3	2.2	±0.7

[1]Determined by drying in forced air oven at 45°C.
[2]Maturity codes: #1 = milk stage; #2 = dough stage; #3 = dent stage; #4 = ripe stage.

of corn silage follows approximately a normal distribution with a slight skewness toward lower dry matter values.

b. Cellulose

The mean cellulose content of corn silage as indicated by Table 31 was 6.6% which is considerably lower than the mean cellulose content of grass silage on an as-fed basis (10.3%). Cellulose content of corn silage, grass silage and hay on an air-dry basis has been discussed in the section "Results and Discussion (Hay)," particularly in reference to its relationship to crude fiber analysis.

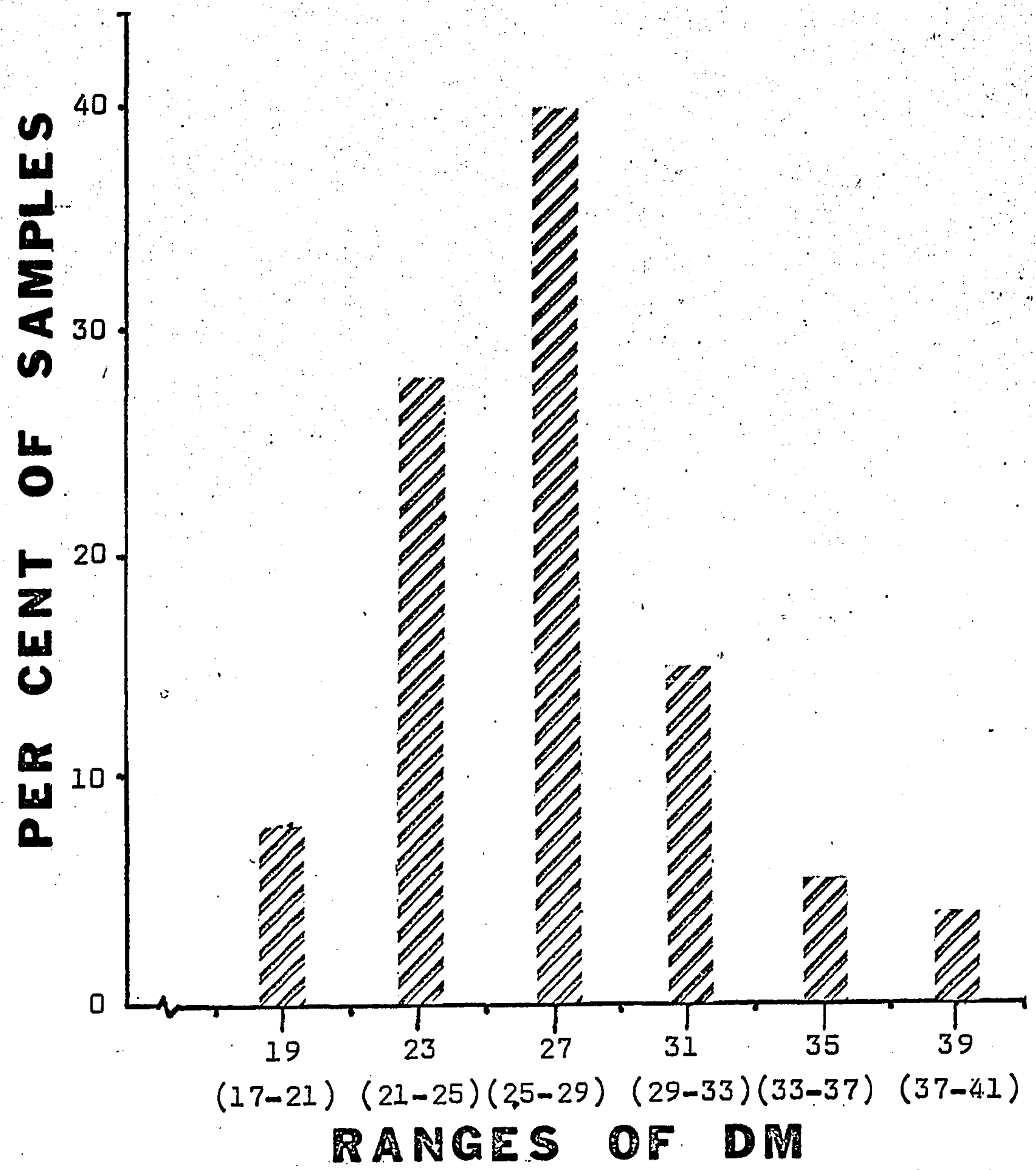

Fig. 7. Distribution of 75 corn silage samples by per cent dry matter.

c. Protein

A mean protein content of 2.0% for corn silage on an as-fed basis clearly indicates the relatively low-protein content corn silage. When the average corn silage protein content is converted to a dried basis by multiplying by the conversion factor $(\frac{100}{26.6})$ the mean protein content of corn silage becomes 7.2%. Thus at a protein content of 7.2% on an equivalent dry matter basis, corn silage still contains considerably less protein than the average for first-cut hay (9.0%; Table 9, page 100).

2. <u>Code data</u>

a. Maturity code

The mean maturity code (Table 31) of 2.2 suggests that on the average, corn silage was harvested at a slightly drier stage than the dough stage which had a maturity code of 2. In Table 32, the distribution of the 75 corn silage samples according to maturity code can be observed. As suggested above, more than half (54.6%) of the corn silage samples had a maturity code of 2. The smallest percentage of silage samples occurred in maturity code #4 (ripe stage). The mean per cent dry matter (DM) for each maturity code indicated that

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TABLE 32. Distribution of 75 corn silage samples by maturity codes

Maturity Code	Number of samples	Per cent of total	Per cent mean dry matter
Code #1 (milk)	10	13.3	23.1
Code #2 (dough)	41	54.6	25.8
Code #3 (dent)	22	29.3	28.8
Code #4 (ripe)	2	2.6	36.8

maturity codes on the average did, in fact, separate the corn silage samples by DM content (or vice versa). As was expected, the more mature codes were the highest in DM. Based on mean DM content of corn silage samples for each maturity code it would appear that the greatest difficulty in assigning maturity codes was to decide whether corn silage was in the dough stage or hard dent stage (codes #2 and #3). The mean per cent DM for each maturity code is in agreement with data presented in "Joint United States-Canadian Tables of Feed Consumption (1960)."

3. <u>Calculated ENE and coded ENE</u>

a. Means compared

Estimated net energy (ENE) was calculated by converting TDN (Penn. State formula) to ENE by Moore's formula, which is the practice followed by the Penn. State Forage Testing Service. Coded ENE values were also assigned to all corn silage samples by the method recommended in the New York (Cornell) DHIA Supervisor's Handbook (1962). These ENE calculated by the two methods are listed in Table 33 along with ENE codes assigned according to the system used by the Macdonald College Dairy Herd Analysis Service (D.H.A.S.).

Although the ENE content of corn silage by the Penn. State and New York methods had almost identical standard deviations the ENE of the former was significantly lower than the latter.

Mean D.H.A.S. (Macdonald College) codes are 10 units lower than Cornell codes because the D.H.A.S. codes were established by reducing each tabular Cornell Code by 10. This reduction was instigated by the D.H.A.S. because it was felt that the nutritive contribution of corn silage was over-rated by the Cornell codes.

In view of the common practice of estimating the

TABLE 33. Comparison of three methods of determining ENE (dry matter basis)

Penn. State		Cornell Codes (D.H.I.A.)		Macdonald Codes (D.H.A.S.)	
Mean	S.D.	Mean	S.D.	Mean	S.D.
61.4[a]	±1.3	64.9[b]	±1.4	54.9[c]	±1.4

[a,b,c]Means with similar superscripts are not significantly different ($P < .01$).

nutritive value of corn silage from its dry matter content, various indicators of nutritive content were compared by ranges of DM (Table 34).

(i) Protein

Protein tends to be slightly higher at the lower DM ranges, and is in agreement with the work of Nehring and Laube (1958) and Johnson and McClure (1968). However, these lower ranges are represented by only a few samples, therefore, it is doubtful if there are any differences in protein at the different DM contents. This range of protein was well within the range of 7.1 to 14.2% reported by Nehring and Laube (1958).

TABLE 34. Influence of dry matter on the average nutritive content of corn silages (dried basis[1])

DM Ranges	Mean DM	Per cent Protein	Per cent Cellulose	TDN[2]	ENE[2]	ENE[3]
17-21	19.1	7.3	32.8	62.9	55.9	57.0
22-25	23.1	8.1	26.4	62.8	55.7	58.5
26-29	26.8	7.7	23.2	63.3	56.4	59.7
30-33	30.8	7.2	23.8	63.6	56.9	61.1
34-37	34.6	7.6	24.0	63.3	56.5	62.0
38-41	39.1	6.8	24.2	63.9	57.2	62.0

[1]Determined by drying in forced air oven

[2]Calculated by Penn. State Formulas

[3]Based on Cornell Quality Codes

(ii) Cellulose

Cellulose (dried basis) shows a trend towards decreasing as the DM of corn silage increases which is in close agreement with the work of Johnson and McClure (1968) who reported significant decreases in cellulose with increasing DM content. Adams and Baylor (1960) observed a similar relationship between crude fiber and DM. These workers suggested that the higher crude fiber content at the low DM ranges was due to relatively high seepage and/or fermentation losses, since any reductions in soluble constituents would result in a percentage increase in insolubles such as cellulose and crude fiber.

(iii) Total Digestible Nutrients (TDN)

TDN by the Penn. State formula show only a trend towards a higher TDN at higher ranges of DM. Adams and Baylor (1960) observed a much more pronounced increase (56.2%-68.0%) in TDN as DM increased from under 20 to 40%. In contrast to this work is the work of Huber _et al._ (1963) which showed no change in TDN on a DM basis for corn silage with a DM content of 25 to 33%. The data in Table 32 clearly reveal only slight differences of TDN within ranges of 26 to 41% DM. However, Johnson and McClure (1968) observed only slight decreases in digestibility of DM and organic matter with advancing maturity, which would produce increasing DDM content with increasing DM levels. Since DDM and TDN are closely related (Hardison 1959), then increased TDN content should parallel increased DM content.

(iv) Estimated Net Energy (ENE)

Calculated ENE values (Penn. State method) show a slight trend to higher ENE values at higher DM ranges. The differences in the pattern of TDN and ENE are due to the effect of the weighting factor of the Moore formula used to convert TDN to ENE (page 88). It is interesting to note in Table 34 that ENE by Cornell Codes begins where the Penn. State ENE values finished. There is a more pronounced increase in coded ENE as DM increases because Cornell ENE values were assigned on this basis; viz., the assigned ENE for 20% DM is lower than the assigned ENE for 40% DM.

b. Correlation coefficients compared

(i) Estimated Net Energy (ENE)

The simple correlation relationships between chemical analyses, maturity code and methods of determining ENE are indicated in Table 35. ENE (Penn. State) is significantly correlated with dry matter (DM), and this relationship is due to the effect of Moore's TDN to ENE conversion equation as was discussed above in relation to Table 34. Correlation coefficients of ENE vs. protein or ENE vs. cellulose (used to predict crude fiber) are not shown because TDN was calculated directly from these chemical analyses and hence the correlation relationships would only confirm this relationship. ENE and maturity code show a small positive correlation

TABLE 35. Simple correlations of chemical analyses and codes of corn silage (75 samples)

Variables Compared	Dry Matter	Protein	Cellulose	Maturity Code	ENE Cornell Code
ENE[a]	0.366**	-	-	0.214	0.363**
Dry Matter	-	0.226	-.417**	0.515**	0.954**
Protein		-	-.101	0.132	0.205
Cellulose			-	-.244	-.466**
Maturity Code				-	0.461**

** $P < .01$

[a]Calculated from TDN (Penn. State formula) by Moore's formula.

coefficient as a result of the significant correlation between DM and maturity code. Estimated Net Energy (Penn. State) was significantly correlated (r = .363) with ENE (Cornell Code), although this relationship was not as high as might be expected, since both ENE values are supposed to be a measure of the same energy component.

(ii) Dry Matter

The low correlation between DM and protein confirms the lack of association between protein and DM as discussed in connection with Table 34, which indicated protein content was fairly constant at the different DM levels. The significant negative relationship between DM and cellulose (dried basis) is due to a greater proportion of low cellulose containing grain in the corn silage with a higher DM content. This relationship is in agreement with data from the "Joint United States - Canadian Tables of Feed Composition" (1964) which show a higher crude fiber content for corn stalks than for cobs with grain. The relationship between DM and maturity code (Table 35) was expected because both parameters are used to indicate maturity. When DM was correlated with ENE (Cornell Code) the resultant high correlation coefficient was anticipated since Cornell Codes were assigned on the basis of DM content.

(iii) Protein

The low correlation coefficient observed between protein and cellulose (Table 35) indicated a trend for protein to increase as cellulose decreased. Protein also shows a low correlation with maturity code indicating a slight increase of protein with advancing maturity. This relationship confirms the relationship between protein and DM. The observed relationship between protein and ENE (Cornell Code) is a direct result of the highly significant correlation between DM and ENE (Cornell Code).

(iv) Cellulose

Cellulose and maturity code reveal a low negative correlation coefficient, which is due to the ears of the corn plant making up a greater proportion of the plant with advancing maturity (Johnson et al. 1966b). Cellulose and ENE (Cornell Code) are significantly correlated as a result of the very close association of Cornell Code and DM and has been discussed above in reference to Table 34.

(v) Maturity code

The significant correlation coefficient between maturity code and ENE (Cornell Code) assigned on the basis of DM content is the result of the observed high association between maturity code and DM. It is of interest to note that ENE (Penn. State) although predicted from crude protein and crude fiber, is significantly correlated with DM and hence substantiates the method of assigning ENE codes on the basis of DM content (page 94).

VI. SUMMARY

A. Hay

1. Differences between first-cut and second-cut (aftermath) hays

Significant differences between first-cut hay and second-cut hays with respect to stage of maturity (page 81), protein content, cellulose content, and NVI were observed. These differences were confirmed by the significant correlation relationship between these criteria. Significant differences between first-cut and second-cut hays were also indicated by mean TDN and ENE contents as determined by both Penn. State methods.

The observed differences between first-cut hay and second-cut hay were generally due to the effect of species (kind). The species effect was first indicated in this study by the results (Table 2) where 13.2% first-cut hay and 80.8% second-cut were coded as 2/3 or more legumes (Kind #1). The effect of species is confounded with the stage of maturity and weather code which would tend to make the species (kind) effect more pronounced (Table 3). The significant effect of plant species on mean protein and NVI content of first-cut hay can be observed with legumes or mixtures high in legumes showing significantly higher values (Table 10). Similar species effects were observed for the 40 selected hay samples (Table 11). There were also significant differences in the mean ADF and cell contents due to legumes (Kind #1).

The effects of species (kind) were observed in the correlation relationships of first-cut, 40 selected and total number of samples. These data clearly indicate the differences between first- and second-cut hays are actually species differences and are in agreement with the work in the literature reviewed.

2. <u>Relationships between cellulose and crude fiber</u>

The early work of Crampton and Maynard (1938) pointed out the close relationship between crude fiber and cellulose (Crampton and Maynard 1938). More recently the Joint United States-Canadian Tables of Feed Composition (1964) have included both crude fiber and cellulose content as indicators of the fibrous portion of feedstuffs. Thus the prediction equations reported herein, with correlation coefficients between crude fiber and cellulose varying from 0.91 to 0.99 could be anticipated. The closest association between cellulose and crude fiber was observed for corn silage. The number of samples used to establish the cellulose-crude fiber relationship was relatively small, particularly for grass silage and corn silage samples.

3. Relationship between NVI and soluble cell contents

The highly significant correlation relationships between NVI and cell contents (Table 16) and confirmed by the multiple correlation relationships (Table 19) indicate the similarity between the methods used by Donefer *et al.* (1966) and Van Soest and Wine (1967) to determine the soluble fraction of forage dry matter. Donefer *et al.* (1966) used an aqueous solution of pepsin-HCl to dissolve the dry matter while Van Soest and Wine (1967) used a neutral detergent solution.

4. Methods of determining Estimated Net Energy (ENE)

It is well recognized that the methods used to determine ENE are not as accurate as *in vivo* Net Energy or *in vivo* TDN values, but some accuracy had to be sacrificed in favour of methods which were rapid and relatively inexpensive. However, the application of basic concepts of Net Energy and TDN is not without source of error. The shortcomings of both the Net Energy system and TDN system were pointed out by Armstrong (1960) and Blaxter (1964) while Maynard (1953) indicated the empirical errors in TDN.

When ENE of the 40 selected samples was determined by five methods, the mean ENE by all methods except the Penn. State - 1 formula method were close (Table 23). The reason mean ENE content by NVI and Van Soest are not significantly different is probably due to the relationship between NVI and soluble cell contents as discussed above.

Cornell Quality code was more closely related to observed cutting date (Table 25) than any of these other parameters used as criteria to measure the nutritive value of forages. This was expected since Cornell codes were assigned on the basis of tabular cutting dates.

NVI is more closely related to ENE by Penn. State - 3 formulas because in these formulas protein has a greater influence on the predicted value than in the Penn. State - 1 formula method and protein accounts for

45% of the variation of NVI (Table 17). Neither protein nor cellulose account for a large percentage of the variation of ENE (Van Soest) or Cornell Quality Codes, therefore ENE by both Penn. State methods which are calculated from protein and crude fiber, are not closely related to ENE by the former methods.

5. Precision of methods of determining Estimated Net Energy (ENE)

According to the data presented (Table 28) the ENE content of hay can vary by ±5 units without necessitating any changes in the farmer's feeding practices. This statement is based on the fact that the average hay consumption of 28 D.H.A.S. herds during the winter-early spring months (January-April 1968) was 9 kg. per cow per day. The data in Table 29 indicate that the observed hay consumption (28 D.H.A.S. herds) for the four months (January-April) was well above the average hay consumption of 5.5 kg. (204 D.H.A.S. herds) for the year.

When a variation of 5 units of ENE is considered permissible for practical purposes it was observed (Table 23) that mean ENE values of four of the methods (Penn. State - 3 formulas, NVI, Van Soest, Cornell Code) of determining ENE are within the desired range. However, the question of which method of determining ENE provides the more accurate evaluation of all samples must also be answered.

6. Sample standard deviation (S.D.) by different methods of determining ENE

The mean S.D. of the 40 selected samples as coded by two D.H.A.S. supervisors (Table 26) was approximately half the S.D. of ENE as determined by Penn. State - 1 formula, Van Soest and Cornell Quality Codes (Table 23). If the S.D. by the majority of methods studied could be considered the more accurate, then ENE by Penn. State - 3 formulas and the D.H.A.S. showed minimal variation whereas ENE as indicated by NVI presents maximal variation. However, if the effect of second-cut hay (species) on NVI (Fig. 5) was eliminated, then the bulk of 1966 and 1967 first-cut hays would fall within the ranges of 23 to 52 as compared to a range of 23 to 62 when the second-cut hay was included. When the S.D. of NVI for first-cut hay was observed (Table 9) it was ± 6.6 and hence close to the S.D. of four of the methods used to determine ENE (Table 23).

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On the basis of the data presented it would appear that D.H.A.S. supervisors do not allow enough variation in their coding and hence all coded values are very close to their mean ENE value. However, the mean ENE content of the forty selected samples as coded by D.H.A.S. supervisors is as precise as the other mean ENE values obtained by Penn. State - 3 formulas, Van Soest, Cornell Quality Code, and NVI methods since they are within the minimum range (±5 units of ENE) within which testing is unnecessary and a tabular value could be used to evaluate forage. Due to the lack of variation of ENE as determined by D.H.A.S. supervisors (Table 26) and predicted by the Penn. State - 3 formulas method (Table 23) the major portion of the ENE values determined by these two methods would be within ±5 ENE units of their respective means. This means that a tabular ENE value equal to the mean ENE values as determined by D.H.A.S. supervisors or the Penn. State - 3 formulas method would evaluate the major portion of the samples with the precision (±5 ENE units) if these methods of evaluating forages are accurate measures of ENE.

B. Grass Silage

1. Protein Content

Although the number of samples of grass silage was limited (24) the data suggest that grass silage when dried has all the characteristics of hay except protein content. The increased protein content of dried grass silage over hay (Table 30) was probably due to less leaf loss due to the ensiling of green material.

C. Corn Silage

1. Relationship of dry matter content and Estimated Net Energy (ENE)

The relationship between dry matter (DM) and Estimated Net Energy (ENE) as indicated by their means (Table 34) shows that as DM content of corn silage increases, the ENE content remains relatively constant on a DM basis. An equal weight of corn silage at the recommended DM content (33% DM) would supply more ENE than wet corn silage (20% DM) due to the diluting effect of water. In addition, Huber *et al*. (1963) and Johnson and McClure (1968) have shown that the voluntary intake of corn silage DM per 100 kg. bodyweight increased as the DM content of the corn silage increased, so more ENE would be consumed by the animal fed corn silage at 33% DM than when corn silage at 20% DM was fed. Both of these observations would contribute to a higher feeding value of 33% DM corn silage.

VII. CONCLUSIONS

In summary of the data herein presented the following conclusions can be made:

1. The differences observed between first-cut and second-cut hays were basically due to differences in plant species (legumes vs. grasses).

2. Crude fiber content of hay, grass silage and corn silage can accurately be predicted from cellulose content of the same forages.

3. The relationships between NVI and ENE (Van Soest) are due to the relationship between NVI and soluble cell contents and acid detergent fiber.

4. Under practical farm conditions in Quebec methods of determining ENE may vary by as much as 5 units without affecting the accuracy of feeding practices.

5. NVI can be used to indicate the ENE of first-cut Quebec hays and can serve as a guide for D.H.A.S. personnel in evaluating Quebec hays.

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5. NVI can be used to indicate the ENE of first-cut Quebec hays and can serve as a guide for D.H.A.S. personnel in evaluating Quebec hays.

6. More crude protein can be obtained from hay crops by ensiling these crops as grass silage.

7. The easiest way for the farmer to improve the nutritive value of his corn silage is to harvest it at the optimum dry matter content.

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