# THE RELATION OF CERTAIN END PRODUCTS OF RUMEN FERMENTATION TO FORAGE FEEDING VALUE

Thesis for the Degree of Ph. D.
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Richard Warren Rice
1960

This is to certify that the

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The Relation of Certain End Products
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Feeding Value

presented by

Richard Warren Rice

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# THE RELATION OF CERTAIN END PRODUCTS OF RUMEN FERMENTATION TO FORAGE FEEDING VALUE

by

Richard Warren Rice

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

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Department of Animal Husbandry
1960

Approved: J. A. Hoefer

# ABSTRACT

Oat straw and alfalfa-bromegrass hay were compared by conventional total digestible nutrient determinations, by *in vitro* volatile fatty acid production and cellulose digestibility.

The alfalfa-bromegrass hay was found to have an average of only 2.4% more TDN than oat straw. Four Holstein cows voluntarily consumed an average of 23 pounds of alfalfa-bromegrass hay per head daily; whereas, a similar group of 4 Holstein cows would consume an average of only 14 pounds of oat straw per head daily. On *in vitro* rumen fermentation alfalfa hay produced volatile fatty acids at a significantly ($p < .05$) faster rate than oat straw. The acetic to propionic acid ratio was ($p < .05$) significantly lower for alfalfa-bromegrass hay for the first four hours, but not significantly different for the balance of the 24 hour *in vitro* fermentation. The percent cellulose digestion on *in vitro* fermentation of alfalfa-bromegrass hay and oat straw was not significantly different.

Differences in ratio and amount of volatile acids produced were significant only when innocula for the fermentations were obtained from steers fed the same forage as that being used for *in vitro* substrate.

The volatile fatty acid production (amount and ratio) and cellulose digestion of holocellulose and cellulose prepared from alfalfa-bromegrass hay and oat straw was not significantly different although early in the fermentation period cellulose digestion and volatile fatty acid production tended to be greater

Richard W. Rice

ABSTRACT (Continued)

when innoculum fermented familiar substrate. Holocellulose and hemicellulose produced a significantly ($p < .05$) lower ratio of acetic to propionic acid than whole forages or cellulose prepared from whole forages. The digestibility of the cellulose of holocellulose and cellulose prepared from the intact forage was significantly ($p < .05$) greater than the digestibility of cellulose in the intact forage.

Although the *in vitro* method utilized was able to distinguish between alfalfa hay and oat straw with respect to quantitative volatile fatty acid production the results on proportions of volatile fatty acids produced are considered inconclusive. The proportions of volatile fatty acids produced after 8 hours or longer of *in vitro* fermentation appear to be more characteristic of the fermentation system than of the substrate being fermented. A short term *in vitro* fermentation of less than 8 hours appears to be necessary for a differentiation between forages on the basis of proportions of volatile fatty acid.

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DISCUSSION

# INTRODUCTION AND HYPOTHESIS

The problem of feed evaluation, especially forages, has occupied the time of many nutritionists for as long as the science of nutrition has existed.

Conventional methods of determining TDN, starch equivalent, or net energy values have worked reasonably well in relation to concentrates but have been less than satisfactory in the case of forages which are useful largely in the nutrition of ruminants. Ruminants are unique in having a digestive system which can derive energy from complex plant carbohydrates. This ability is due to the symbiotic relationship of bacteria and protozoa with the ruminant. The microflora carried in the rumen enables ruminants to derive energy from complex plant carbohydrates since they readily hydrolyze these materials into compounds which the animal can utilize.

Most methods of feed evaluation depend on the results of digestion trials which are difficult and expensive. The application of the results from a few animals with a few feed samples to all animals and plants of varying age, species, plane of nutrition and enviroment leads to considerable error. It would be useful if a simpler test could be devised which could be used to evaluate feeds individually for various species of animals under varying conditions, so that the errors inherent in one evaluation based on specific conditions when applied to a large variety of cases could be minimized. The evaluation of a forage based on its fermentation by rumen microbiota may be more logical than conventional methods since a large part of the energy made available from forage is a result of the degradation of ingested food material by ruminal microflora.

It has been noted for many years that the heat increment of feeds is higher in ruminants when feeds containing relatively high proportions of lignin and fiber are consumed. This higher heat increment is not accounted for by conventional TDN computations nor by starch equivalent or net energy evaluations even when a fiber correction as suggested by Kellner (1920) is used. Recent work by Armstrong, Blaxter and co-workers (1956, 1957a, 1957b) has indicated that the heat increments produced by the three volatile fatty acids (acetic, propionic and butyric), which are the major end products of rumen microbial dissimilation of carbohydrates, may be different.

Many other workers have shown that the type of carbohydrate fermented influences the relative proportions of volatile fatty acids which are produced. From the preceding it may be reasoned that the nutritive value of a forage as a source of energy depends on the rate, extent and proportions of volatile acids produced by rumen microbial fermentation.

The objectives of the research reported here were:

(a) To test the hypothesis that the nutritive value of a forage depends on the rate and extent of and the proportions of volatile fatty acids produced by rumen microbial fermentation.

(b) To develop a simpler more accurate method for forage evaluation.

# REVIEW OF LITERATURE

## Forage Evaluation

### The importance of energy:

McCullough (1959) determined that in the case of forages the nutrients least likely to be limiting were crude protein and minerals. He concluded that the limiting factor as far as forages are concerned was the level of energy. In a summary of 70 forages based upon analyses taken from the literature, he found no correlation between crude protein and metabolizable energy content, while very high correlations occurred between crude fiber and dry matter digestibility (-.908), crude fiber and metabolizable energy (-.932) and dry matter digestibility and metabolizable enrgy (.986). He had found earlier (McCullough, 1956) that dry matter intake and digestibility were highly correlated.

In a similar series of experiments and calculations Crampton (1957) concluded that relative to the average TDN which forages yield, protein, calcium and phosphorus tend to be present in forages in concentrations equal to or greater than those indicated by feeding standards to be necessary for cattle maintenance. Energy was the fundamental limiting factor in the nutritive value of a forage. In a test using five forages of varying quality he found that digestible calories and protein content were significantly correlated. Lignin content was positively correlated with voluntary consumption. No significant correlations were found between voluntary consumption and the

digestibility of dry matter, protein, fiber or calories. However, the correlations between dry matter consumption and the digestibility of dry matter, protein and calories were all nearly significant. The high (.98) positive correlation between lignin content and dry matter intake was explained on the basis that the effect of lignin on digestibility is due to its distribution in the plant and not to its absolute content. The data illustrate that absolute content of lignin cannot be used as a measure of forage voluntary consumption. Crampton proposed that the rate of digestion and forage intake are inhibited by anything which represses the microfloral activity of the rumen. He suggested that a practical numerical rating of forages could be evolved by expressing the voluntary daily consumption of a forage as a percentage of a "normal" or "expected" value of 3.0 lb. (dry wt.) per 100 lb. live weight of animal.

Blaxter (1956) states that shortages of dietary energy are usually a more important cause of low productivity in farm animals than dietary deficiencies of other nutrients.

Forbes _et_ _al_. (1928) determined that if a feed fed to cows was rated on the basis of 1.00 for maintenance, the net energy utilization was .985 for lactation and only .761 for body increase in energy. Thus, nutritive value depends on the function for which nutrients are used.

Blaxter (1956) concluded that, with respect to ruminants, "The key to food evaluation lies in the elucidation of the chemical, physical and biological factors which control the bacterial population and the kinetics and energetics of the dissociation of complex carbohydrates by ruminal microflora."

Deficiency of present systems of forage evaluation:

Reid *et al.* (1959) suggested that present standards or indices are not very precise in predicting forage value. He agreed with Crampton (1956) and McCullough (1959) that forage quality depends largely on the rate of consumption and energy content of the forage. He also noted that forages, even of the same species, may be greatly different with respect to the response they can produce, but that conventional data do not reflect the magnitude of these differences. He suggested a rating system for forages based on the voluntary dry matter intake and energy content of the forage.

Blaxter (1950, 1951) states that the theoretical upper limit to production occurs at a level of intake which is beyond the animals' feed capacity. He suggests that the indigestible residue of lower quality feeds has an effect on feed capacity. Therefore, for animals on high production, the value of a feed of low nutritive worth is less than can be inferred from conventional analyses.

Kleiber (1959) concluded that "No single reference unit is adequate for feed evaluation for growth and milk production." He criticizes the use of TDN, net energy, and Scandinavian feed units on the basis that these evaluate protein as a source of energy, not as protein *per se* which is best utilized for milk production or muscle tissue. He proposed a dual reference substance with two constants, one for energy replacement and the other for protein replacement value when related to a standard substance. The standard substances being a combination of common feeds such as cottonseed meal and barley.

Forbes and Voris (1932) determined that cows receiving chopped hay transformed 18.3% of the TDN in the feed to milk TDN. When an alfalfa hay-grain ration was fed 22.7% of the feed TDN was transformed to milk TDN.

Elliot and Loosli (1959) determined that TDN and digestible energy values overestimate the available energy in roughages and underestimate the available energy in concentrates when applied to ruminants.

Huffman and Duncan (1944) determined that an equal TDN replacement of alfalfa hay with corn or wheat markedly increased milk production. They theorized that cereals contained a "lactation factor" which caused increased milk production.

Smith _et_ _al_. (1945) determined that cows did not produce as well as expected from the TDN intake of alfalfa hay supplemented with salt and phosphorus. Twelve different concentrates fed alone or with starch or sugar gave superior production when replacing approximately equal amounts of the TDN of alfalfa hay. The production from alfalfa was not quite as high as expected when the adequacy of the ration was calculated on the basis of net energy.

Saarinen _et_ _al_. (1951) recalculated the net energy values of hay according to Kellner's (1900) method and concluded that Huffman's (Huffman and Duncan, 1944) grain lactation factor was simply an energy discrepancy. They fed cows according to their net energy requirements from 56 to 154 days on alfalfa alone or in combination with purified carbohydrates or carbohydrates plus fat. No abnormal decrease in milk yield occurred. When corn was used in place of pure substances no efficiency changes occurred.

Shaw (1959) noted that when good quality roughages are evaluated using the TDN or digestible energy systems the discrepancy between TDN and actual net feeding value is not great. However, in the case of poor quality roughages, roughage TDN may have no more than 50% as much available energy as TDN produced from concentrates or good quality roughages.

Other workers have noted an over-evaluation of roughages by the TDN or net energy system using roughages alone versus roughages plus concentrate on an equal TDN or net energy basis (Nordfeldt _et_ _al_., 1950; Teichman _et_ _al_., 1958; Loosli _et_ _al_., 1955; Irvin _et_ _al_., 1951).

Moore _et_ _al_. (1953) determined that the increased milk production when concentrates replaced part of an all hay ration on an equal TDN basis could be explained on the basis of high net energy content of concentrates. They worked out a series of equivalences between TDN and net energy. These were: 1 lb. TDN in corn = 1 Therm net energy, 1 lb. TDN in good hay = .75 Therm net energy and 1 lb. TDN in poor roughage = .50 Therms of net energy. From these calculations it is apparent that when poor quality roughages are used their net energies are as little as 1/2 the amount indicated by the TDN content. Even TDN from good quality hay supplies only 3/4 as much net energy as an equivalent amount of TDN from corn. These differences must be due largely to the extra heat increment and increased amount of combustible gases produced from the digestion and metabolism of roughages as compared to concentrates, since these losses are not considered in TDN and digestible energy calculations.

## In Vitro Fermentation

### Application to feed evaluation:

The *in vitro* method of investigation into rumen function has become popular recently due to the difficulties involved in assessing rumen volumes and absorption of end products of fermentation. However, a wide variety of techniques have been developed, many of which have serious limitations (for discussion, see Warner, 1956), the largest one being certainty that conditions are reasonably representative of those *in vivo*. One of the major difficulties involved is that *in vivo* conditions are not well defined and are very complex. Thus the question of criteria for validity of *in vitro* work is a difficult one to answer.

Louw *et al.* (1949) showed that the accumulation of end products during *in vitro* fermentation slowed the rate. However, no difference was noted in the proportions of volatile acids produced compared to *in vivo* observations. Gray and Pilgrim (1950, 1952) and Gray *et al.* (1951) noted that the composition of volatile fatty acids observed *in vitro* contained a greater proportion of propionic acid than rumen fluid. The extent to which the various carbohydrates were digested was similar to the rumen breakdown. They hypothesized that the differences observed *in vitro* and *in vivo* were due to the differential absorption of volatile acids.

Cheng *et al.* (1955), using a washed cell technique, investigated several variables as related to cellulose digestion. The rate of cellulose digestion was much slower *in vitro* than that observed *in vivo*. Substrate concentration and pH had a great influence on rate and extent of cellulose digestion *in vitro*. Pigden and Bell (1955) describe an *in vitro* fermentation system which, when used with prediction equations developed by them, gave estimates of TDN and digestible crude protein for eleven forages which were in close agreement with those derived from conventional procedures. Barnett (1957) proposes an *in vitro* method for determining the digestibility of cellulose. His results correlated reasonably well with digestion trials.

Baumgardt *et al.* (1958) compared several laboratory methods of TDN estimation with those determined *in vivo*. Cellulose digestion determined by *in vitro* rumen fermentation was most closely related to *in vivo* digestion. The relationship was not significant when results from all the forages tested were compared, however, a highly significant correlation occurred when only grass hays were considered. Brown *et al.* (1958) determined that *in vitro* digestibilities reflected *in vivo* observations though they were not identical. A 24 hour fermentation (*in vitro*) was suggested by Kamstra *et al.* (1958) as a measure of cellulose availability.

Heuter *et al.* (1958), using the washed cell technique of *in vitro* fermentation, concluded that it was useful in outlining or elucidating one or two step reactions but observations involving multi-step reactions were of doubtful significance unless verified _in vivo_. Asplund _et al_. (1958), employing an _in vitro_ method utilizing the dialysing sac technique of Huhtanen _et al_. (1954), showed distinctly different percentages of dry matter were lost and significantly different amounts of fatty acids produced when feeds of diverse quality were fermented. There was a high degree of correlation between dry matter digestibility _in vivo_ and dry matter loss and fatty acid production _in vitro_.

Using a different approach Leffel (1958) found that the relative proportions of rumen volatile fatty acids did not change though a 20% increase in VFA was noted when rumen contents were incubated _in vitro_ for 2 hours. Longer fermentation periods led to definite discrepancies in proportions of VFA produced. A similar method was used by Stewart _et al_. (1954) to estimate rate and amounts of volatile fatty acid production. His results agreed reasonably well with observations determined by different techniques.

Quicke _et al_. (1959) obtained good agreement between _in vitro_ and _in vivo_ digestibilities with seven grass hays. However, results were not consistent with the six legume hays which were investigated. An inhibitory substance to _in vitro_ cellulose digestion was reported by Dehority (1957) to be present in alfalfa extracts which may be related to the discrepancies noted when legumes are tested _in vitro_.

Church and Peterson (1960) investigated the influence of several variables on _in vitro_ rumen fermentations. They noted that a wide variety of *in vitro* systems is in use today, each with its characteristic results, which makes interpretation difficult and between laboratory comparisons of little value. However, they concluded that the use of the laboratory fermentation for feed evaluation and as a research tool holds great promise, pending standardization of technique.

McBee (1953) describes a manometric method for the evaluation of rumen microbial activity. Its use is limited since the proportions of the various volatile fatty acids are not estimated, total acid production only being determined.

In general the *in vitro* rumen fermentation technique is able to yield results on dry matter and cellulose digestibilities which correlate well with *in vivo* observations. The volatile fatty acid production, however, is not as well correlated with *in vivo* results. The discrepancy may be due to an alteration of the fermentation due to the experimental conditions or to the effect of ruminal absorption of acids on the volatile acids present in the rumen. Since there is no real evidence that differential absorption of volatile fatty acids from the rumen exists, (most evidence indicates that differential absorption of the volatile fatty acids does not occur, Annison *et al.*, 1957; Pfander and Phillipson, 1953; McCarthy *et al.*, 1958; Davis *et al.*, 1958; Barcroft *et al.*, 1943; Conrad *et al.*, 1956; Gray, 1947; Gray, 1948; Stewart *et al.*, 1958), the tendency is to believe that the differences observed in volatile fatty acids present in *in vitro* fermentation flasks is due to an alteration in the fermentation imposed by experimental conditions.

Source of innoculum:

Gall *et al.* (1949) cultured cattle and sheep rumen bacteria from animals on various diets. They concluded that the bacteria present were similar in both species. However, the number of fast growing organisms increased when high concentrate rations were fed.

Burroughs *et al.* (1950) reported that the kinds and numbers of bacteria cultured from rumen contents differed with corn cob-alfalfa rations with and without starch and with varying amounts of casein. Fast growing species were more evident when starch was present in the ration. Hunt *et al.* (1952) made a similar observation. Hungate *et al.* (1952) noted rapid changes in ruminal flora when large amounts of grain were placed in sheep rumens which had previously been fed an all alfalfa hay ration.

In 1953 Bryant and Burkey compared the microflora from cows fed a variety of diets. Larger numbers were cultured on the all-concentrate ration than on other rations. The flora cultured from a cow receiving wheat straw was least complex, that from a concentrate fed cow intermediate, and that from a cow fed alfalfa hay the most complex. The numbers of starch and cellulose digesters varied directly with the amounts of starch and cellulose contained in the ration. The level of feeding of rations did not effect numbers or types of bacteria cultured.

Maki and Foster (1957) counted directly and cultured greater numbers of bacteria from rumen fluid of cows receiving high concentrate rations than in rumen fluid from roughage fed cows. The number cultured from a high roughage rumen fluid was only 3-12% of the total direct count. In contrast the cultural count of high concentrate rumen fluid was 57-73% of the direct count. The roughage fermenting bacteria had much more fastidious nutritional requirements than concentrate fermenting bacteria. They grew only when rumen fluid was included in the culture tubes. They grew better in mixtures, pure isolates growing poorly or not at all.

These observations would indicate that cultural methods for counting or isolating roughage type micorflora may lead to serious errors of estimate. Thus colony counts as an enumerating device should be interpreted cautiously especially when roughage type microflora are being used. Further, the ration probably influences the development of a ruminal microflora characteristic of that ration.

## The Importance of the Rumen Fermentation

### Extent of digestion in the rumen:

As long ago as 1883 Tappeiner (cited in Annison and Lewis, 1959) demonstrated that the fermentation of cellulose in the rumen of the ox resulted in the formation of large amounts of volatile fatty acids (VFA's). For many years little nutritional significance was placed on this observation. The digestion of carbohydrates in the rumen was believed to be a depolymerization to simple sugars which were then absorbed and metabolized in the same manner as in monogastric animals (Annison and Lewis, 1959). It is now established that the VFA's found in the rumen constitute a major source of energy to the ruminant since little of the digestible carbohydrate escapes rumen fermentation.

Hale et al. (1947), using the lignin ratio technique, determined that the rumen digestion of cellulose was 78.9%, dry matter 84.9%, protein 86.3%, nitrogen-free extract 100.2%, crude fiber 58.0%, other carbohydrates 101.6% and lignin 14.4% of the fecal digestion of the above fractions.

Gray (1947) studied the progress of cellulose digestion along the gut of sheep. An average of 42.7% of dietary cellulose was degraded by the time digesta had reached the abomasum and 58.8% by the time the colon was reached. Thus 70% of the digested cellulose was digested in the rumen-reticulo-abomasal complex, 17% in the caecum and 13% in the colon. Gray et al. (1951b) incubated wheaten and alfalfa hays in vitro and found the yield of volatile acids to be 1/4 of the substrate or 50% of the digestible matter.

Weller and Gray (1954) used the lignin ratio technique with rumen and abomasal fistulae to show that the destruction of starch in the rumen and omasum was nearly complete. Starch passing through the abomasum increased from 1 gram per day when 3 grams were fed to only 8 grams when 148 grams were fed.

Balch and Rowland (1957) in an extensive investigation of the effects of several variables on the production of volatile fatty acids noted that very little carbohydrate which could be digested in the lower digestive tract escaped rumen fermentation. Paloheims et al. (1955) showed that of the total amount of the nitrogen-free, non-lignin organic matter digested in the whole gut, 76-99% was digested in the reticulo-rumen and omasum. When Balch (1957) used the lignin ratio technique to estimate rumen digestion he found that 43% of the dietary dry matter was digested in the reticulo-rumen. This represented 81% of the fecal digestion of NFE and 54% of the fecal digestion of crude fiber. Ninety-three to ninety-five per cent of the starch was digested in the reticulo rumen. Gray _et al._ (1958) determined that an average of 40% of the cellulose and 43% of the pentosans was digested in the stomach of sheep. Kameoka and Morimato (1959) observed that 61.7 to 85.4% of the digestible organic matter disappeared from the fore-stomach of goats. Meyer _et al._ (1959) killed sheep at 0, 1½, 4 and 8 hours after feeding and found that 89 per cent of the holocellulose was digested by the time it reached the abomasum. Rogerson (1958) estimated that 40% of the dietary dry matter was digested in the rumen of sheep fed hay compared to 50% on a mixed diet and 75% on a concentrate ration. The total dry matter (fecal) digestibilities were 49, 62 and 88% respectively.

Consequently, it is apparent that the energy made available to ruminants is largely the result of rumen fermentation. A major end product of this fermentation is the volatile fatty acids. Therefore, any consideration of the energy nutrition of ruminants involves a consideration of these acids and their utilization.

Importance of volatile fatty acids:

The composition of the volatile acids produced on rumen fermentation of carbohydrates was not known until Elsden et al. (1946) worked out a column chromatographic method for their separation. An average of 329 grams of volatile fatty acids was found in the reticulo rumen of cattle and 64 grams in sheep. These acids were about 67% acetic, 19% propionic and 14% butyric. Other workers have observed considerable amounts of volatile fatty acids produced during rumen fermentation (Balch, 1958; Gray et al., 1951b; Phillipson, 1952; El-Shazly, 1952; Annison, 1954).

Carrol and Hungate (1954) estimate that 66% of the energy derived from a ration is from volatile fatty acids. It was shown that 6,000 to 12,000 calories became available in the rumen of cattle every day. Phillipson and Cuthbertson (1956) have calculated from the data of Schambye (1951) that at least 600-1,200 calories of energy are absorbed as VFA's from the sheep rumen every 24 hours. Stewart et al. (1958) estimated from rate determinations on rumen fluid of steers fed several rations, that the volatile fatty acids produced in 24 hours would furnish from 12,000 - 13,000 calories representing 37.9% of the digested calories of the ration. This would account for 63% of the maintenance requirements of the steers. McCarthy et al. (1957) using a rumen perfusion technique estimated that rumen VFA would provide 37 to 46% of the energy requirement of the goat for maintenance. Emery (1956) estimated, from turnover curves determined in vivo, that a cow would obtain 3-13% of its energy from the volatile fatty acids.

## The Relation of the Diet to the Volatile Fatty Acids

### The components of the diet:

The percent of milk fat in the milk of cows is positively correlated with the proportion of acetic acid in the rumen. Powell (1938, 1939) first observed that the fat content of milk decreased when cows were fed low roughage rations. Not until 10 years later was the depression of milk fat linked with alterations in the proportions of volatile fatty acids occurring in the rumen.

Stoddard et al. (1949) observed that low roughage diets caused a lowering of the proportion of acetic acid in the rumen accompanied by a lowered fat test in milk. When acetic acid was given by stomach tube the milk fat test returned to normal. Propionic acid administered in the same manner had no effect on the fat content of milk. These observations were confirmed by Tyznick and Allen (1951) and Van Soest and Allen (1959). Rook and Balch (1959) raised milk fat test in milk from cows receiving normal rations by acetate infusion. Propionic acid infusions had no effect on milk fat test.

Gray et al. (1952) established the presence of formic, acetic, propionic, n-butyric, isobutyric, valeric, caproic and probably heptoic acid in rumen fluid. They determined the molecular proportions of fatty acids in the rumen fluid 1 to 2 hours after feeding. Lucerne hay gave values of 66.9% acetic, 22.5% propionic, 6.7% butyric, and 3.2% valeric acids. The averages of 7 observations from sheep fed wheaten hay were 65.8% acetic, 22.1% propionic, 9.2% butyric and 2.2% valeric acid.

Card and Shultz (1953) determined that grain added to hay or pasture rations caused a decrease in the proportion of acetic acid. The proportion of propionic acid on the hay plus grain ration was decreased slightly but a large increase in butyric acid occurred. When grain was fed on pasture there was a fall in acetic acid proportion accompanied by an increase in butyric and propionic acids. The lowest proportion of acetic acid occurred when an all concentrate ration was fed. Roughages from later dates of cutting tended to cause an increase in acetic and a decrease in butyric acid. Silage tended to cause a decrease in acetic and an increase in propionic acid when compared with hay. Acetic acid proportions were the most variable with reciprocal changes in butyric acid.

Hibbs _et_ _al_. (1954) fed several diets with various hay-grain ratios to calves and noted that with higher grain levels the proportion of butyric was increased with a reciprocal decrease in the propionic acid proportion of total volatile fatty acids. Reid (1957) determined that the pre-feeding proportion of propionic and butyric acids in the rumen of sheep increased as the level of concentrate in the ration increased. The proportion of acetic acid in the rumen decreased when Balch and Rowland (1957) decreased the ratio of hay to concentrate in a diet fed to cows.

Johns (1955) observed that the proportion of acetic acid dropped markedly when sheep were changed from hay to pasture. There was also a marked rise in the butyric acid proportion, that of propionic remaining fairly constant. A higher proportion of acetic acid was observed when hay of poorer quality was fed. A great consistency of rumen volatile fatty acids occurred in the rumen of sheep consuming pasture herbage which covered a wide range in maturity and chemical composition. In contrast to Reid (1957), Jamieson (1959) observed, _in_ _vivo_, that sheep grazing normal pasture showed seasonal fluctuations in the proportions of ruminal volatile fatty acids. Samples containing low proportions of acetic acid were found in the spring and fall when lush pastures occurred. The conclusion was that seasonal variation of volatile fatty acids in sheep on pasture were due to variations in plant composition. When sheep were dosed with nitrate and nitrite at sub-lethal levels, decreases in the proportion of acetic with concomittant increases in the proportion of propionic and butyric acids occurred. This was hypothesized to be due to a competition between acetate production and nitrate reduction for hydrogen donors.

Elliot _et_ _al_. (1957) observed _in_ _vivo_, increased rumen acidity and higher total volatile acids with a greater proportion of propionic accompanied by decreased amounts of butyric and acetic acids when corn silage as sole roughage was compared to hay crop silage as sole source of roughage. Rumen acidity was not increased by either hay-crop or corn silage when they were fed as a part of the roughage ration.

The *in vitro* volatile fatty acid production from various feeds was studied by Stewart and Schultz (1958). Urea consistently increased volatile fatty acid production regardless of substrate. In a subsequent *in vivo* experiment urea increased VFA production only slightly; however, the level of urea achieved in the rumen was not as great as that in the *in vitro* experiments. Fresh, hand-clipped legume mixed grass caused a greater VFA production than legume hay. The grass markedly depressed propionic acid compared to hay. Molasses decreased acetic acid production, beet pulp increased acetic acid production and corn meal increased propionic acid production.

Van Soest and Allen (1959) found no change in rumen acetic acid on low roughage diets, but an increase in the molar percent of propionic acid occurred. They calculated that a significant negative correlation occurred between the fat content of milk and proportion of propionic acid in the rumen. When they fed 1 lb. of acetate per day, the depression of milk fat was corrected. Propionate feeding tended to cause a further decline in milk fat percent.

Elliot and Loosli (1959) determined that the relative concentration of propionic acid in rumen liquor was highly correlated with both the gross and net production efficiency. Negative correlations existed between the acetic/propionic ratio and gross and net efficiencies. The molar proportions of acetic and propionic acids were closely related to the average crude fiber content of the ration (negatively for propionic and positively for acetic acid.) The net efficiency of utilization of digestible energy was highly correlated (negatively) with the crude fiber content of the ration.

## Components of plants and their relation to VFA production:

The wide variety of volatile fatty acid proportions formed from different feeds by ruminal fermentation has led to the search for a particular component of these feeds which is responsible for the production of acetic, propionic and/or butyric acids.

Elsden (1945), in an *in vitro* fermentation of glucose, observed that propionic acid was the predominant acid produced. When dried grass was fermented, acetic acid was the predominant acid produced. The *in vitro* fermentation of xylose and glucuronic acid produced preponderating amounts of acetic acid (Heald, 1952). Marston (1948) noticed a high proportion of propionic acid when cellulose was fermented *in vitro*.

Gray and Pilgrim (1952a, 1952b) examined *in vitro*, the volatile fatty acids produced from cellulose and hemicellulose alone, in combination, in the presence of wheaten hay or in the presence of a small amount of protein prepared from wheaten hay. The greatest proportions of acetic and butyric acids and least proportion of propionic acid occurred when wheaten hay was the substrate. Cellulose, hemi-cellulose or a combination of cellulose and hemi-cellulose produced a lower proportion of acetic and butyric acids and a higher proportion of propionic acid. When 4% wheaten hay protein was added to the cellulose-hemicellulose mixture the portion of propionic acid decreased and those of acetic and butyric acids increased over that observed when no protein was added to the mixture. When wheaten

hay was mixed with the pure components, the proportions of acids produced on in vitro fermentation were intermediate between those of the separate substrates. Casein in the place of wheaten-hay protein did not affect acetic acid proportions, however, the butyric acid proportion increased greatly accompanied by a fall in the proportion of propionic acid.

Phillipson (1952) noted that the proportion of acetic acid in a fermentation mixture increased with increasing fiber content. However, in over 400 in vivo determinations of the individual volatile fatty acids of sheep consuming pasture at various stages of maturity, Johns (1955) observed no marked change in total volatile fatty acids or in acid proportions. Although there was considerable variation in the chemically defined constituents of the pasture herbage ingested they appeared to bear no definite relation to the proportions of volatile fatty acids occurring in the rumen. Jamieson (1959) observed decreases in acetic and increases in propionic and butyric acid proportions when lush pasture was consumed. However, during the remainder of the season relatively small fluctuations occurred in volatile fatty acid proportions.

Barnett and Reid (1956) determined in vitro that the amounts of acetic acid produced diminished in the order: dried grass, cellulose, glucose and lactic acid. The more easily fermented the food the less the proportion of acetic acid produced. When fresh grass was fermented at various stages of maturity the propionic acid proportion of the volatile fatty acids became greater as the grass became more mature. In 1957 Barnett and Dowe deterthat the fermentation of pyruvic acid produced a high proportion of acetic acid. Propionic acid was the chief acid derived from lactate. They proposed a metabolic scheme for the formation of propionic acid via succinic acid and the citric acid cycle.

Barnett and Reid (1957), using an *in vitro* technique, studied the production of volatile fatty acids obtainable from dried grass and its grass water-soluble and water-insoluble separates. In contrast to fresh grass, dried grass gave a consistently greater proportion of propionic acid at all stages of maturity. Aqueous extracts yielded preponderating amounts of acetic acid while the residue gave amounts of propionic acid equal to or exceeding the corresponding production of acetic acid. Commercial cellulose produced propionic acid in the largest proportion. Following this up (Barnett and Reid, 1957b) they found that crude fiber and cellulose, prepared from dried grass, produced proportions of the volatile fatty acids between those of water extracted dried grass and commercial cellulose. They concluded that the major portion of acetic acid comes from the carbohydrate fractions in whole grass which are simpler than cellulose and that the proportions of acetic acid observed *in vivo* were difficult to account for in regards to *in vitro* determinations.

Hershberger *et al.* (1956), obtained *in vitro*, a preferential formation of acetate when pyruvate formed 50% of the carbon of the substrate. Lactate as 50% of the carbon of substrate led to the preferential formation of propionic acid. Glucose, pyruvate, lactate and malate increased both acetic and propionic acid production while malonate, glutamate and $\alpha$-Keto glutaric acid increased

acetic acid production. Jayasuriya and Hungate (1959), using radioactive labeling, determined that lactate was not an important intermediate in hay fed animals. Lactate $-2-C^{14}$ was recovered chiefly as acetic acid when allowed to ferment in vitro.

Starch yielded lower proportions of propionic and a higher proportion of valeric, butyric and acetic acids than cellulose in vitro. Xylan and pectin produced the same proportions of volatile fatty acids as starch (Belasco, 1956). Lewis and McDonald (1958) found no striking differences in the volatile fatty acids when they fermented xylan, levan and starch. The volatile fatty acid production from carbohydrate was enhanced in the presence of casein in the rumen of sheep.

The importance of protein to roughage digestion when roughage was fed in the absence of starchy feed was small (Burroughs et al., 1950). When starch was added to a low-protein roughage ration, the dry matter digestibility of roughage dropped drastically; however, when starch and protein were added together the decrease in digestibility due to starch was much less. Crampton (1956) made a similar observation on the importance of protein to roughage digestion from which he concluded that with most roughages nitrogen content was not the first limiting nutrient, the primary limitation to productivity of roughages being energy. Head (1953), on the basis of experimentation, concluded that if a roughage has a minimum content of 1% nitrogen, cellulose digestion was independent of supplementary nitrogen. However, Belasco (1954a, 1954b) determined that in vitro cellulose digestion was best supported by urea supplied up to the 35% protein equivalent level.

Urea as the sole source of nitrogen promoted the production of higher levels of propionic acid and lower levels of butyric and valeric acids than did equivalent amounts of nitrogen from either soybean oil meal, linseed oil meal, cottonseed oil meal or corn gluten meal. The acetic acid and total volatile fatty acid levels were unaffected. He postulated that the additional propionic acid resulted from a high cellulolytic activity in the artificial rumen in the presence of a readily available source of nitrogen.

Annison (1954) noticed that protein induced a greater production of butyric plus higher volatile acids. Others have made similar observations (Otagaki *et al.*, 1955; Woodhouse *et al.*, 1955; Annison, 1956; Davis *et al.*, 1957; El-Shazely, 1956).

## Other Factors Influencing the Rumen Fermentation

### Feed treatment:

It has been found that, in addition to the ration fed, certain treatments of the ration will affect proportions of volatile fatty acids in the rumen, milk fat test, and efficiency of ration energy utilization.

Powell (1939) noted that grinding of hay resulted in depressed milk fat test. These results were confirmed by Balch (1952, 1958) who also noted an increased proportion of propionic acid produced by the fermentation. Pelleted alfalfa hay in lamb diets resulted in increased rate of gain and increased

feed consumption (Neale, 1953; Cate et al., 1955; Esplin et al., 1957). Blaxter and McGraham (1955, 1956) noted that pelleted hay fed to cows passed through the digestive tract at a faster rate, with a lower digestibility and lower metabolizable energy content than chopped hay, but was equal in net energy value to chopped hay because of a lower heat increment. In contrast, Meyer et al. (1959), using a different experimental approach, concluded that pelleted alfalfa hay fed to sheep contained no greater net energy than long hay and that increased gain noted with pelleted hay was due to an increased intake.

The form in which the roughage is fed may also influence relative proportions of volatile fatty acids. Dried grass produced a greater proportion of acetic acid than the same grass fed fresh or preserved as silage (Johns, 1955; Card and Schultz, 1954; El-Shazley, 1952). Barnett and Reid (1957) made the opposite conclusion. From their in vitro observations, dried grass gave greater proportions of propionic acid than fresh grass, however, their in vitro method was of long duration (72 hours) and only terminal observations on volatile fatty acid proportions were made. The results of this type of technique have been seriously questioned (Warner, 1956).

Balch and co-workers (1954, 1955a, 1955b, 1955c) conducted a series of experiments in which they determined that the essential feature of diets which depress milk fat was not simply a low roughage or crude fiber content per se. Low roughage rations did lower fat content, but, the greatest depression of milk fat was obtained with a high starch intake. In addition, the type of starch was also instrumental in lowering the fat content of milk. When flaked maize (heated and rolled corn) was compared with maize meal or barley and oats, the greatest fat depression occurred with the flaked maize followed by the maize meal and the barley and oats ration.

The work of Shaw *et al.* (1959) and Ensor *et al.* (1959) confirms the observations of Balch and co-workers on the effect of cooked grains on milk fat test and proportions of volatile fatty acids present in the rumen. Ground, pelleted roughage alone did not affect the proportions of volatile acids produced or the milk fat test. However, the effect of cooked concentrates was enhanced when they were fed with ground hay instead of long hay.

The efficiency of gain (Ensor *et al.*, 1959) and milk production (Elliot and Loosli, 1959) may be increased when these types of rations are fed.

## The rate of fermentation:

There is some evidence that the rate of the ruminal fermentation may influence the proportions of volatile acids produced.

During periods of rapid acid production Gray and Pilgrim (1951, 1952) found that the ratio of acetic to propionic acid decreased; whereas, when acid production was slow the ratio increased. Carroll and Hungate (1954) observed that the *in vitro* rate of fermentation and the proportion of propionic acid produced was greater when rumen contents were fermented for one hour from a grain fed steer than that produced upon incubation of rumen fluid from an animal fed roughage.

Hershberger et al. (1956) using washed cells to ferment cellulose observed that acetic acid was the major volatile fatty acid produced during the early part of the fermentation when the rate was slow. After 15 hours the rate of fermentation and the production of propionic acid increased. When Belasco (1954b) fermented cellulose he noted a more rapid rate of fermentation and the production of a greater proportion of propionic acid when urea was used as a nitrogen source in place of natural proteins.

When Stewart et al. (1958) fed a mixed diet to cows they observed from rate curves established in vitro that propionic acid was produced at a faster rate than either butyric or acetic acids immediately after feeding. At 10 hours after feeding a prominent increase in the production of acetic acid occurred. The rate of production of volatile fatty acids was greatest immediately after feeding.

Reid (1957) observed in sheep that the proportion of acetic acid declined and that of propionic acid increased after feeding. The lowest proportion of acetic acid and highest proportion of propionic acid coincided with the maximum total VFA levels in the rumen.

In contrast Sheppard et al. (1958) could not find any difference in the proportions of volatile acids present in the rumen of sheep fed hay although variations in the amount of total acids present occurred during 24 hours of fermentation.

The rate of feeding may also affect acetic/propionic acid ratios. Williams and Christian (1956) noted a higher ratio of acetic to propionic acids when they fed a limited amount of hay as compared to liberal feeding.

### The effect of pH:

Balch et al. (1956) found that during rumen fermentation there was a decline in pH after feeding. This decline was greater for low roughage diets than for those high in roughage. The lowest pH occurred at a time when the greatest proportion of propionic acid was observed. The rate of VFA production was maximal at this point. Lactic acid was present during periods of low pH and may have contributed to the pH change. Balch and Rowland (1957) noted that an acid rumen inhibited the production of acetic acid. The acidity resulted from the rapid fermentation of soluble carbohydrates. Elliot et al. (1957) observed decreased pH coinciding with increased proportions of propionic acid when a ration of corn silage was fed as compared to a hay-crop silage ration.

If the depression in pH is too great, a different result may occur. Annison and Lewis (1959) indicate that the normal range of the rumen pH is 5.5 to 7.0 and that those outside of this range often lead to rumen dysfunction. Reid et al. (1957) found that when the pH of the rumen fell below 5.2 due to lactic acid accumulation, the propionic and butyric acid proportions fell. The pH had lowered to the point where rumen function was inhibited greatly.

Energy relationships:

The ruminant animal is recognized as having a higher heat increment than other classes of livestock. This phenomenon was noted by Kellner (1920) when he stated that ruminant heat losses associated with carbohydrate dissimilation were 30 to 70 kilogram calories per 100 kilogram calories of carbohydrate ingested. This is compared to heat lost in man of only 5 to 7 kilogram calories per 100 kilogram calories of carbohydrate ingested. (Lusk, 1928).

The heat of the rumen fermentation has been considered as a source of the higher heat increment in ruminants. However, Marston (1948) fermented cellulose *in vitro* and determined that the heat of fermentation under normal conditions could account for only 15% of the heat increment. Later Marston (1951) concluded that the heat loss occurring in the dissimilation of acetic acid was probably responsible for the relatively high rate of heat production in the ruminant. McClymont (1952) postulated that the high heat increment of acetic and butyric acids could be inferred from the intermediary metabolism of these acids, their oxidation being thermodynamically wasteful. He suggested a specific dynamic effect of 50 to 70% for acetic and butyric acids.

Not until 1956 were there any real attempts to assess the effect of the volatile fatty acids on ruminant heat increment. Armstrong and Blaxter (1956, 1957) infused volatile fatty acids into the rumen of sheep. When only acetic acid was administered 41 to 50% of the energy input appeared as heat. In contrast the heat increment of propionic acid was 13% and that of butyric 16%. The heat increment of glucose infused into a non-fermenting rumen was only 6.4%. In a more extensive study Armstrong et al. (1957) infused various combinations of the volatile acids, acetic, propionic and butyric, into the rumen of fasting sheep. There was no difference in heat increment when the infusion mixture contained from 10 to 90% acetic acid. Some manifestations of ketosis occurred when 100 and 90% acetic acid mixtures were infused. It appeared that small quantities of propionic acid (1 molecule of propionic to 15 molecules of acetic acid) were sufficient to facilitate the oxidation of acetic acid and protein breakdown to form carbohydrate in the fasting animal. The conclusion was that sub-maintenance heat increment was not due to variations in the proportions of volatile acids produced in the rumen.

When volatile fatty acids were infused into the rumen of sheep receiving super-maintenance diets a different relationship was observed (Armstrong and Blaxter, 1957). The heat increment of the various acids when fed alone was 67% for acetic, 33% for propionic and 38% for butyric. The mean heat increment of a mixture containing 75% acetic acid, 15% propionic acid and 10% butyric acid was 68.1%, while that of a 25:45:30 mixture was 41.9%. Any energy retention from the infused acids was assumed to result in increased fat content of the body. Therefore, for fat synthesis the mixture containing relatively high amounts of acetic acid was used much less efficiently than a mixture containing only 25% acetic acid.

# PART I. DIGESTION TRIAL

## Procedure

Eight dry Holstein cows were used to determine the digestibility of alfalfa-bromegrass hay and oat straw[1].

These cows were fed the forages, "ad libitum", for a ten day preliminary period followed by a 7 day fecal collection period. The cows receiving the oat-straw were also fed 2 pounds per head daily of a complex supplement containing protein, vitamins and minerals (Table 1). This supplement was considered necessary in order to keep the cows from going into drastic nitrogen imbalance during the feeding period. Maynard and Loosli (1956) state that the actual digestibility of a feed not sufficient in itself is more truly evaluated when supplements are fed in much the same manner in which they would be fed in actual practice. In addition, in this study, the greatest interest was in differences of energy rather than vitamins, minerals and protein.

During the ten day preliminary period the cows were adjusted to a uniform daily intake. During the collection period daily allowances were kept constant for each cow so that she was consuming uniform amounts of the forage each day. All cows readily consumed the alfalfa-bromegrass hay ration so that several days prior to and during collection 3 cows were consuming 24 pounds per day and a fourth (#3) 20 pounds per day. More difficulty was encountered in getting the oat-straw fed cows to eat uniform

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[1] Provided by Dr. Lassiter of the M.S.U. Dairy Department.

TABLE 1. COMPOSITION OF OAT STRAW SUPPLEMENT

| Ingredients | Percent |
|---|---|
| Ground Shelled Corn | 30.00 |
| Soybean Oil Meal | 32.25 |
| Dried Molasses | 10.00 |
| Dehydrated Alfalfa Meal | 15.00 |
| Dicalcium Phosphate | 5.00 |
| Trace Minerals[1] | .20 |
| Trace Mineralized Salt | 1.50 |
| Vitamin A and D Concentrate[2] | .05 |
| Urea | 6.00 |

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[1] Trace Mineral Pre-Mix, Calcium Carbonate Company, Quincy, Illinois.

[2] 9,800 I.U. Vit. A/gm. 1,250 I.U. Vit. D/gm.

TABLE 2. PROXIMATE ANALYSIS OF FEEDS

|  | Ash | Crude Fiber | Ether Extract | Water | Protein | N-Free Extract |
|---|---|---|---|---|---|---|
| Alfalfa-Bromegrass hay | 5.29 | 29.28 | 1.51 | 10.98 | 14.38 | 38.56 |
| Oat Straw | 5.91 | 38.76 | 1.17 | 10.61 | 3.82 | 39.73 |
| Oat Straw Supplement | 9.36 | 7.50 | 1.70 | 11.21 | 35.56 | 34.67 |

daily amounts. Three of the four, however, were eating the straw readily prior to and during fecal collections at a rate of about 14 pounds per head daily. One of the cows refused the straw for several days and would not consume uniform daily amounts.

Fecal collections were weighed daily, mixed thoroughly, and aliquots, preserved with thymol, stored under refrigeration in air-tight jars. Conventional proximate analyses were carried out on the feed and feces (Table 2).

## Results

The average TDN content of the alfalfa bromegrass hay ration was 64.0%, the range from 63.3% to 65.4%, and for the oat straw ration 57.5% with a range from 45.3% to 62.1% (Table 3). The low value is suspect since this was the value obtained with the cow which would not consume the oat straw readily. If this value is eliminated and the average of the other three determinations calculated, the TDN value for the oat straw ration would be 61.6% with a range from 60.9% to 62.1%.

TABLE 3. TDN VALUES OF RATIONS FED

| Ration | Observation | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Ave. |
| Alfalfa Bromegrass | 65.4 | 63.5 | 63.3 | 63.8 | 64.0 |
| Oat Straw Ration | 61.7 | 62.1 | 45.3[1] | 60.9 | 57.5 |

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[1] This value is probably in error. For explanation, see text.

This study was conducted to demonstrate that TDN and forage feeding value are not always in the same relationship. The results support this contention and led to subsequent experimental investigations reported in Part II.

## PART II. THE FERMENTATION OF OAT STRAW AND ALFALFA-BROMEGRASS HAY

### Procedure

*In vitro* fermentation:

Two mature Hereford steers, equipped with permanent plastic type rumen fistula, were used as sources of innocula in this study. One steer was maintained on oat straw plus 2 pounds of supplement (Table 1), the other on alfalfa hay plus 2 pounds of supplement. Each steer was fed for at least 15 days on their respective ration before being used as innoculum source for *in vitro* fermentations. This was to insure that the microflora of the steers had an opportunity to adjust to the type of ration fed.

On the days when experiments were conducted fluid was drawn out of the rumen before feeding in the morning and strained through two layers of cheese cloth into a warmed thermos, which had been previously gassed with $CO_2$. The innocula were transported to the laboratory where 800 milliters were mixed with substrate (2% by weight substrate) in 950 ml. bottles, which were then placed in a 39°C. water bath and bubbled continuously with $CO_2$ during pipetting. The flask was swirled vigorously before each sac was innoculated to insure mixing of the substrate. To supply an adequate amount of available nitrogen 2 ml. of a solution containing 200 mg. urea/ml. was added to the bottles.

Twenty ml. of the innocula plus substrate solution were pipetted, with a wide orifice pipett, into cellophane dialysing sacs[1] which were then placed in 4 ounce, screw cap bottles containing 80 milliliters of a buffered salt solution warmed to 39°C. with mineral composition approximating that of ruminant saliva (Salsbury et al., 1956). The bottles were closed tightly and transferred to a 39°C. incubator. At predetermined time intervals representative bottles were removed from the incubator, the contents of the sac and bottle mixed and transferred quantitatively to a 200 ml. centrifuge bottle. The mixture was centrifuged 20 minutes at 1200 rpm. A 10 ml. aliquot of the supernatant was made alkaline by adding 1 ml. of normal potassium hydroxide and evaporated to dryness on a steam bath. The dried aliquot was stored at room temperature until analysed.

### Volatile fatty acid determinations:

For VFA analyses the residue from the dried aliquot of the fermentation supernatant was dissolved in 2 ml. of 12% by volume sulfuric acid, mixed with 2 grams of silicic acid[2] and transferred to a chromatographic column described by Keeney (1955).

The column packing material for the chromatographic columns was prepared by mixing silicic acid which had been dried overnight at 175°C. with glycol reagent in proportions of one gram

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[1] Made from cellophane tubing 32/32 Visking Corporation, Chicago, Illinois.

[2] Mallinckrodt's, stock no. 2847.

of silicic acid to 0.9 ml. of reagent. The glycol reagent was made by dissolving 700 mg. of brom cresol green in 700 ml. ethylene glycol on a steam bath. After cooling, 200 ml. of water were added followed by 40 ml. of 0.1 N. ammonia water. Additional water was added to make one liter of solution.

Forty grams of the silicic acid-glycol reagent mixture were slurried with n-hexane[1] and poured into a glass chromatographic column, equipped with a fritted glass disc and tefflon stopcock. The top of the column packing material was leveled with a flattened glass rod and n-hexane was run through the material under 5 to 10 lbs. of nitrogen gas pressure until the column appeared packed uniformly, care being taken to prevent the solvent from sinking below the top of the packing material. The columns were then ready for the addition of the sample section.

After the sample was added to the top of the column packing material the yellow bands indicative of acids were developed with a 1% butanol-hexane solvent which was allowed to run through the column under atmospheric pressure. When the first (butyric plus higher acid) fraction had reached the point of elution the fraction collector was changed. The butyric plus higher acids fraction was then eluted with the 1% butanol-hexane solution.

The solvent was changed to 5% butanol-hexane for the elution of propionic and acetic acids which were collected in separate flasks. The fractions were titrated with .025 N. alcoholic potassium hydroxide using thymol blue as the titration end-point indicator.

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[1] Skelly Solve B, Skelly Petroleum Company.

There were three replicate flasks for each time period. The volatile fatty acids were determined on two of the replicates or three if variation between replicates was greater than 10%. The values reported are the averages of at least two and sometimes three replicates.

Sets of 12 to 18 columns were run simultaneously. Mixtures of pure acetic, propionic and butyric acids were used to determine blank values for the system, appropriate corrections being made where necessary.

No attempt was made to separate butyric acid from higher volatile acids since preliminary work had indicated that good separation of standard mixtures of butyric and valeric acids was not consistently achieved under the conditions in this laboratory. For convenience, in subsequent discussion, the butyric plus higher acids fraction will be referred to as butyric acid.

## Holocellulose determination:

When holocellulose disappearance was determined the procedure of Wise et al. (1946) was used. The precipitate from the centrifugation of fermentation flask contents was transferred to a 200 ml. Berzelius beaker, washing with water, and dried overnight at 105°C.

The dried material was digested under a hood in 16 ml. of water plus 0.15 gm. sodium chlorite and one drop of glacial acetic acid at 70-80°C. in a water bath. After one and two hours of digestion 0.15 gm. sodium chlorite and one drop of glacial acetic acid were added to the mixtures. The digestion was allowed to continue for a total of three hours.

After the three hour digestion the samples were cooled in ice water, transferred to a centrifuge bottle and centrifuged for 20 minutes at 1500 rpm. The supernatent was decanted and the precipitate collected on a weighed fiber glass filter, washed with ice water and acetone, dried for 48 hours in a desiccator and weighed. This weight was taken to be the holocellulose content of the fermentation flask. The difference between amounts present in zero hour samples and those which had been incubated was taken to be the amount of holocellulose digested. The preliminary ether extraction of Wise _et al_. (1946) was omitted. No corrections for ash content were made. Cellulose determinations were carried out on this residue by the Crampton and Maynard (1938) procedure, with modifications, when both holocellulose and cellulose disappearance was estimated.

Difficulty with filtration and lack of agreement of duplicate samples led to the abandonment of the holocellulose determination.

### Cellulose determination:

For determination of cellulose the precipitate from centrifugation of the contents of the fermentation bottle was transferred quantitatively, washing with water, to a 200 ml. Berzelius beaker and dried overnight at 105°C. This material was used in subsequent analyses for cellulose by a modification of the Crampton and Maynard (1938) procedure (Salsbury _et al_., 1956).

Cellulose was determined by adding 15 ml. of 80% (by volume) acetic acid and 1.5 ml. of concentrated nitric acid to each beaker. The mixture was refluxed for 20 minutes on a hot plate. A round-bottom flask filled with cold water served as a condenser. The samples were then filtered through a selas porcelain crucible of extra coarse porosity, washing with absolute ethanol.

The filtrate in the crucibles was dried at 105° for 12 hours, cooled in a desiccator and weighed. Then the samples were ashed at 700°C. for two hours, cooled and weighed a second time. The difference in weight before and after ashing was taken to be the cellulose content of the sample. The benzene and ether washes of the Crampton and Maynard method were omitted. The difference between the amounts of cellulose in the zero hour samples and the amounts in the incubated samples was considered to be the amount of cellulose digested.

Cellulose determinations were accomplished on three replicate fermentation flasks for each time period of fermentation. The values reported represent averages of the three determinations.

## Experimental design and reporting of data:

The experiment was a 2 x 2 factorial design. There were four treatments, two with each type of innoculum. These were:

(1) Innoculum from an alfalfa[1] fed steer with alfalfa as fermentation substrate (AA),

(2) Alfalfa innoculum with oat straw as substrate (AO),

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[1] For convenience the alfalfa-bromegrass hay will be referred to as alfalfa hay.

(3) Innoculum from an oat straw fed steer reacting with alfalfa as substrate (OA), and

(4) With oat straw as substrate (OO).

Time periods of fermentation were 2, 4, 8, 12 and 24 hours. Each experimental treatment was repeated two separate times. The values reported are the averages of the results of the two trials.

Statistical tests were conducted using the analysis of variance technique (Snedecor, 1956).

The values reported for volatile fatty acid production were obtained by subtracting the micro-equivalents present in the fermentation flasks at zero time from the amounts present after fermentation. The amounts of volatile acids produced due to the substrate carried over in the rumen fluid were measured on several occasions. After 2 - 4 hours of fermentation the rate of production had become very slow with little net volatile fatty acids being produced past the 8th hour of fermentation. The practice of obtaining rumen fluid approximately 14 hours after the last feeding is probably responsible for there being relatively little fermentable substrate carried over in the rumen fluid innocula. In addition Asplund *et al.* (1958), using a similar technique, concluded that the fermentation occurring in the absence of substrate was different from that occurring in the presence of substrate. Therefore, the volatile fatty acid values reported represent the difference from zero hour. They are expressed as micro-equivalents of acid per fermentation flask.

## Results

<u>Volatile fatty acid production</u>:

Table 4 summarizes the cumulative production of the volatile fatty acids on the various treatments. Figure 1 is a visual projection of the data for total volatile acid production. The amounts of volatile fatty acids produced are expressed in micro-equivalents of acid above that present in the original sample. Analysis of variance (Snedecor, 1956) indicates that alfalfa innoculum and substrate produced a greater quantity $(p. < .05)$ of total volatile fatty acids than oat straw innoculum or substrate. Treatment AA gave the highest volatile acid production with OO giving the lowest total volatile fatty acid production $(p < 0.05)$. The volatile fatty acid production on treatments OA and AA were intermediate and not significantly different.

The results indicate that there was a real difference in the production of volatile fatty acids when oat straw was compared to alfalfa hay. These differences were most evident when the substrate tested was fermented with an innoculum obtained from an animal which had been receiving that substrate as a major portion of his ration. The volatile fatty acid production when alfalfa innoculum reacted with oat straw (AO) and oat straw innoculum reacted with alfalfa substrate (OA) was intermediate between treatments AA and OO.

The average ratio of acetic to propionic acid produced during the entire fermentation period was not significantly different for any treatment. The highest ratio was obtained

## TABLE 4. THE CUMULATIVE *IN VITRO* PRODUCTION OF VOLATILE FATTY ACIDS

| Period | Fraction[1] | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | OO | | OA | | AO | | AA | |
| | | Amt. | Ratio | Amt. | Ratio | Amt. | Ratio | Amt. | Ratio |
| 0-2 Hrs | 1 | 30 | | 12 | | 16 | | 24 | |
| | 2 | 6 | 17.33 | 14 | 6.28 | 30 | 3.73 | 44 | 5.36 |
| | 3 | 104 | | 88 | | 112 | | 236 | |
| | Total | 140 | | 114 | | 158 | | 304 | |
| 0-4 Hrs | 1 | 30 | | 28 | | 40 | | 70 | |
| | 2 | 16 | 9.13 | 32 | 6.13 | 62 | 3.84 | 116 | 3.74 |
| | 3 | 146 | | 196 | | 238 | | 434 | |
| | Total | 192 | | 256 | | 340 | | 620 | |
| 0-8 Hrs | 1 | 54 | | 54 | | 88 | | 144 | |
| | 2 | 58 | 4.31 | 80 | 4.25 | 160 | 2.90 | 256 | 2.84 |
| | 3 | 250 | | 340 | | 464 | | 728 | |
| | Total | 362 | | 474 | | 712 | | 1128 | |
| 0-12 Hrs | 1 | 74 | | 98 | | 132 | | 172 | |
| | 2 | 102 | 3.86 | 160 | 3.39 | 264 | 2.44 | 328 | 2.87 |
| | 3 | 394 | | 542 | | 644 | | 940 | |
| | Total | 570 | | 800 | | 1040 | | 1440 | |
| 0-24 Hrs | 1 | 126 | | 196 | | 196 | | 320 | |
| | 2 | 234 | 2.89 | 360 | 2.86 | 420 | 2.37 | 634 | 2.41 |
| | 3 | 676 | | 1030 | | 996 | | 1530 | |
| | Total | 1036 | | 1586 | | 1612 | | 2484 | |

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[1] Fraction 1 = butyric + higher acids; 2 = Propionic acid; 3 = Acetic Acid.

with the oat straw innoculum-substrate treatment (OO) and the lowest with the alfalfa innoculum-oat straw substrate (AO) treatment. The effect of innoculum source was nearly significant.

The differences in ratio were widest during the early part of the fermentation. The OO treatment produced very little propionic acid during the first two hours of fermentation as is indicated by the high ratio (17.33) observed. From the second to the fourth hour the production of propionic acid became appreciable and the ratio after 4 hours of fermentation was lowered to 9.13. After 8 hours of fermentation the ratio decreased further to 4.31.

There was a tendency for the ratio on all treatments to decline indicating that the production of propionic acid became relatively more important as the fermentation progressed.

Analysis of variance of the observations for each time period indicate that alfalfa innoculum produced a significantly (p .05) lower ratio of acetic to propionic acid up to 4 hours of fermentation and that after this time there was no significant difference in ratio although alfalfa innoculum tended to produce a lower ratio throughout the fermentation.

A relatively high ratio of acetic to propionic acids was observed after 2 hours of fermentation on all treatments. This appears to be characteristic of roughages fermented under this system. In a preliminary experiment a small amount of concentrate was added to the fermentation flasks. After two hours of fermentation a low ratio of acetic to propionic acid was observed. This was accompanied by a very rapid rate of fermentation. The

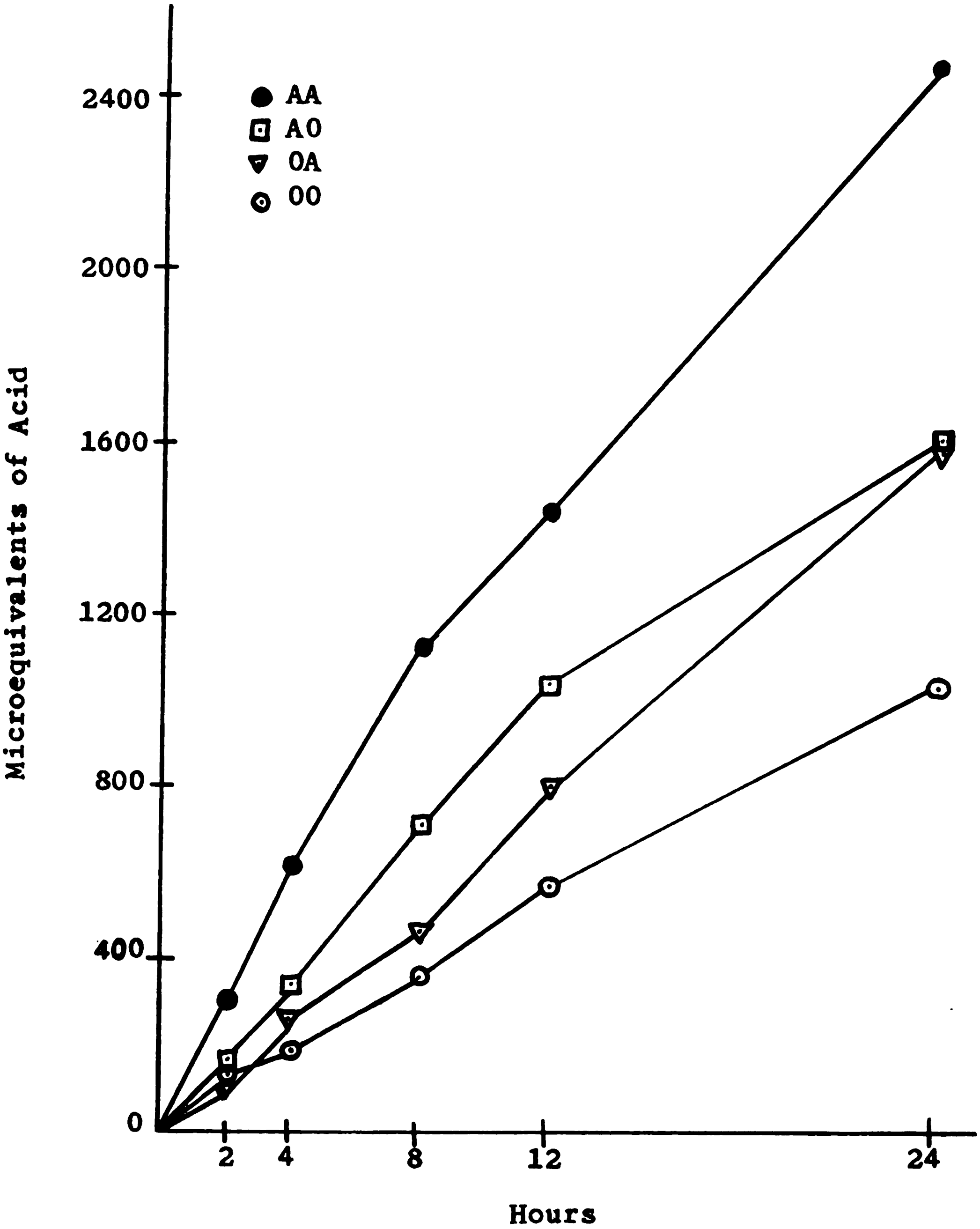

FIGURE 1. THE CUMULATIVE IN VITRO PRODUCTION OF VOLATILE FATTY ACIDS

early rapid volatile acid production and low ratio observed when concentrates were supplied indicate that they were readily attacked by the rumen microflora. Further, their fermentation led to the formation of proportionately greater amounts of propionic acid than roughages.

The digestion of cellulose, holocellulose and hemicellulose:

In some cases the precipitate from centrifugation of the contents of the fermentation flasks was analysed for holocellulose, hemicellulose and cellulose. The results using these methods were so variable and the method so cumbersome that they had to be abandoned. It was noted, however, that the digestion of holocellulose, hemicellulose and cellulose in whole plant material proceeded at about the same rate.

Table 5 gives the results of the disappearance of holocellulose, hemicellulose, and cellulose during a preliminary experiment using oat hull holocellulose and alfalfa innoculum. The disappearance of hemicellulose was determined by difference so that much of the error involved in holocellulose and hemicellulose determinations appears in this fraction. Little degradation of either substrate appeared to take place before the 8-12 hour fermentation period. The production of volatile fatty acids was also slow until this period. This would indicate that oat hull holocellulose was attacked slowly under the conditions of this experiment. The degradation of cellulose proceeded to a greater extent (59%) than hemicellulose (46%). Due to the variation involved in the determinations, this difference could hardly be considered significant. However, it is apparent that

TABLE 5. OAT HULL HOLOCELLULOSE FERMENTATION BY FERMENTATION PERIOD

| | Fraction[1] | 0-2 Hrs. | 2-4 Hrs. | 4-8 Hrs. | 8-12 Hrs. | 12-24 Hrs. | 0-24 Hrs. |
|---|---|---|---|---|---|---|---|
| Microequivalents Net VFA[2] | 1 | 000 | --- | 86 | 76 | 134 | 304 |
| | 2 | 60 | --- | 12 | 164 | 228 | 460 |
| | 3 | 68 | --- | 98 | 130 | 348 | 614 |
| | Total | 128 | --- | 196 | 370 | 710 | 1378 |
| Ratio[3] | | 1.13 | --- | 8.17 | .79 | 1.53 | 1.33 |
| Percent Holocellulose Disappearance/pd | | 3.6 | 4.2 | .00 | 27.9 | 17.1 | 50.6 |
| Percent of Cellulose Disappearance | | 6.0 | 1.0 | .00 | 21.0 | 30.0 | 59.0 |
| Percent Hemicellulose Disappearance[4] | | 0.0 | .00 | 3.0 | 33.0 | 10.0 | 46.0 |

[1] Fraction 1 = butyric plus higher; 2 = propionic; 3 = acetic acid.

[2] Microequivalents of acid produced during the time period.

[3] Ratio obtained by dividing the meq. acetic acid by meq. propionic acid produced during the period.

[4] Obtained by difference between holocellulose and cellulose present.

hemicellulose, when it is in combination with cellulose as holocellulose, is degraded at about the same rate and to about the same extent as cellulose.

The greatest amount of hemicellulose (33%) disappeared during the 8th to the 12th hour of fermentation while cellulose was degraded appreciably during both the 8th to 12th hour (21%) and 12-24th hour (30%). During the period when the bulk of hemicellulose disappeared the relative proportion of propionic acid produced as indicated by the low ratio (.79) was greatest. This would indicate that hemicellulose, under the conditions of this experiment, was fermented with the production of relatively more propionic acid than was obtained from cellulose. The results on fermentation of the various plant fractions, discussed in Part III tend to support this conclusion.

The disappearance of cellulose on the various treatments is summarized in Table 6. The results are expressed as the weight (milligrams) of cellulose and as the percent of total cellulose which disappeared during the time period considered.

The oat straw appeared to contain cellulose which was more resistant to attack than alfalfa cellulose. During the first 2 hours of fermentation on treatment 00 there was no apparent disappearance of cellulose while on treatment A0 only 1.7% of the digestion occurred. This is compared to 4.8% and 2.4% disappearance on treatments AA and 0A. During the first 8 hours of fermentation an average of only 6.4% of the cellulose had disappeared when oat straw was the substrate. In contrast

## TABLE 6. CELLULOSE DIGESTION BY FERMENTATION PERIODS

| Period | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 00 | | OA | | AO | | AA | |
| | Wt(mg) | | Wt(mg) | | Wt(mg) | | Wt(mg) | |
| 0 Hour | 164.2 | | 144.4 | | 145.0 | | 97.6 | |
| | Wt Dig | % Dig | Wt Dig | % Dig | Wt Dig | % Dig | Wt Dig | % Dig |
| 0-2 Hour | 00 | 00 | 3.4 | 2.4 | 2.5 | 1.7 | 3.9 | 4.8 |
| 2-4 Hour | 4.3 | 2.6 | 9.0 | 6.2 | 2.8 | 2.0 | 6.7 | 6.9 |
| 4-8 Hour | 4.2 | 2.6 | 10.7 | 7.4 | 5.7 | 3.9 | 7.8 | 7.9 |
| 8-12 Hour | 13.7 | 8.3 | 11.0 | 7.6 | 20.2 | 13.9 | 6.5 | 6.7 |
| 12-24 Hour | 43.9 | 26.7 | 34.3 | 23.8 | 25.1 | 17.3 | 12.0 | 12.3 |
| 0-24 Hour | 66.1 | 40.2 | 68.4 | 47.4 | 56.3 | 38.8 | 36.9 | 38.6 |

17.4% of the cellulose had disappeared after eight hours of fermentation when alfalfa hay was the substrate. Over the total fermentation period oat straw innoculum was able to degrade a greater proportion of the cellulose present (43.8%) than alfalfa innoculum (38.7%). In every case the total amount of cellulose digested was greatest from the 12th to the 24th hour of fermentation.

The weight of cellulose digested was appreciably less in treatment AA than in the other treatments. However, this may be due to the low (97.6 mg.) level of cellulose contained in these flasks compared to that on the other treatments. The amount of digestion late in the fermentation period was less on this treatment than other treatments which contained greater total amounts of cellulose. This indicates that the concentration of substrate may have limited the disappearance of cellulose.

# PART III. THE FERMENTATION OF ALFALFA AND OAT STRAW HOLOCELLULOSE, HEMICELLULOSE AND CELLULOSE

## Procedure

There is some reason to believe that there is a difference in the structure of cellulose from different plant materials (Baker *et al.*, 1957). The distribution and amount of lignin also has been implicated in differences in forage utilization (Crampton, 1957). Consequently, alfalfa and oat straw holocellulose, hemicellulose and cellulose were prepared by the methods of Wise *et al.* (1946) to investigate these factors. These were subjected to the same experimental treatments as the whole plant in a 2 x 2 factorial design. The production of the volatile fatty acids and the disappearance of cellulose were measured.

## Results

### *The production of volatile fatty acids from the holocellulose of alfalfa hay and oat straw:*

The volatile fatty acid production during each fermentation period from holocellulose prepared from alfalfa hay and oat straw is summarized in Table 7. There were no significant differences in the total amounts of volatile fatty acids produced nor were there any differences in the ratios of acetic to propionic acid due to treatment during the 24 hours of fermentation.

There was a tendency for volatile acid production to be greater when innoculum fermented holocellulose prepared from the forage to which it was accumstomed (treatments AHA, alfalfa innoculum-alfalfa holocellulose and OHO, oat straw innoculum-

## TABLE 7. IN VITRO VOLATILE FATTY ACID PRODUCTION ON HOLOCELLULOSE SUBSTRATE BY FERMENTATION PERIODS.

| Period | Fraction[1] | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | OHO | | OHA | | AHA | | AHO | |
| | | Amt.[2] | Ratio | Amt. | Ratio | Amt. | Ratio | Amt. | Ratio |
| 0-2 Hrs | 1 | 24 | | 26 | | 80 | | 28 | |
| | 2 | 22 | 3.18 | 24 | 2.50 | 94 | 2.83 | 44 | 2.36 |
| | 3 | 70 | | 60 | | 266 | | 104 | |
| | Total | 116 | | 110 | | 440 | | 176 | |
| 2-4 Hrs | 1 | 36 | | 44 | | 46 | | 30 | |
| | 2 | 68 | 3.56 | 60 | 3.37 | 52 | 2.35 | 32 | 3.12 |
| | 3 | 242 | | 202 | | 122 | | 100 | |
| | Total | 346 | | 306 | | 220 | | 162 | |
| 4-8 Hrs | 1 | 28 | | 36 | | 58 | | 42 | |
| | 2 | 64 | 2.59 | 72 | 3.11 | 112 | 1.93 | 136 | 1.29 |
| | 3 | 166 | | 224 | | 216 | | 176 | |
| | Total | 258 | | 332 | | 386 | | 354 | |
| 8-12 Hrs | 1 | 60 | | 40 | | 52 | | 54 | |
| | 2 | 228 | 2.26 | 100 | 2.52 | 150 | 1.31 | 216 | 1.42 |
| | 3 | 516 | | 252 | | 196 | | 308 | |
| | Total | 804 | | 392 | | 398 | | 578 | |
| 12-24 Hrs | 1 | 34 | | 28 | | 148 | | 34 | |
| | 2 | 310 | 0.32 | 294 | .81 | 312 | 2.18 | 210 | .40 |
| | 3 | 98 | | 238 | | 682 | | 84 | |
| | Total | 442 | | 560 | | 1142 | | 328 | |
| 0-24 Hrs | 1 | 182 | | 174 | | 384 | | 188 | |
| | 2 | 692 | 1.58 | 550 | 1.77 | 720 | 2.06 | 638 | 1.21 |
| | 3 | 1092 | | 976 | | 1482 | | 772 | |
| | Total | 1966 | | 1700 | | 2586 | | 1598 | |

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[1] Fraction 1 = Butyric plus higher acid; 2 = propionic acid; 3= acetic acid.

[2] Amounts expressed as microequivalents acid per 100 ml rumen fluid equivalent.

oat straw holocellulose).

The greatest amount of volatile acids was produced by the AHA treatment followed by the OHO treatment. The cross-over treatments produced fewer total acids with the oat straw innoculum-alfalfa holocellulose (OHO) treatment slightly superior to the alfalfa innoculum-oat straw holocellulose (AHO) treatment. This is an indication that there may be a basic difference in the holocellulose of alfalfa hay and oat straw.

In contrast to the fermentation of the whole forage the average ratio of acetic to propionic acid tended to be highest on the AHA treatment which produced the greatest amount of volatile fatty acids and lowest on the AHO treatment which produced the least amount of volatile acids. This indicates that the higher average ratios observed upon the fermentation of oat straw as compared to alfalfa hay may be due to factors other than holocellulose. The ratios of acetic to propionic acid produced after the first 4 hours of fermentation were much lower than those observed when the whole forage was fermented. All except the AHA treatment were less than 1 during the last 12 hours of the 24 hour fermentation.

The very low ratios of acetic to propionic acid observed during the last 12 hours of fermentation on treatments OHO, OHA and AHO were accompanied by a slowed rate of fermentation. However, the AHA treatment which had a higher (2.18) ratio for the last 12 hours of fermentation produced a considerably greater quantity of volatile acids than the other treatments during this period. It is possible that the slowing of the fermentation rate on the other three treatments may have had an influence in causing the low ratios at that time.

The volatile acid production during the early stages of fermentation was greater for alfalfa innoculum than for oat straw innoculum as was observed during the fermentation of whole alfalfa hay and oat straw. It was also greater when innoculum fermented holocellulose prepared from the same forage fed to the animal which served as an innoculum donor.

## Cellulose disappearance on the fermentation of the holocellulose of alfalfa hay and oat straw:

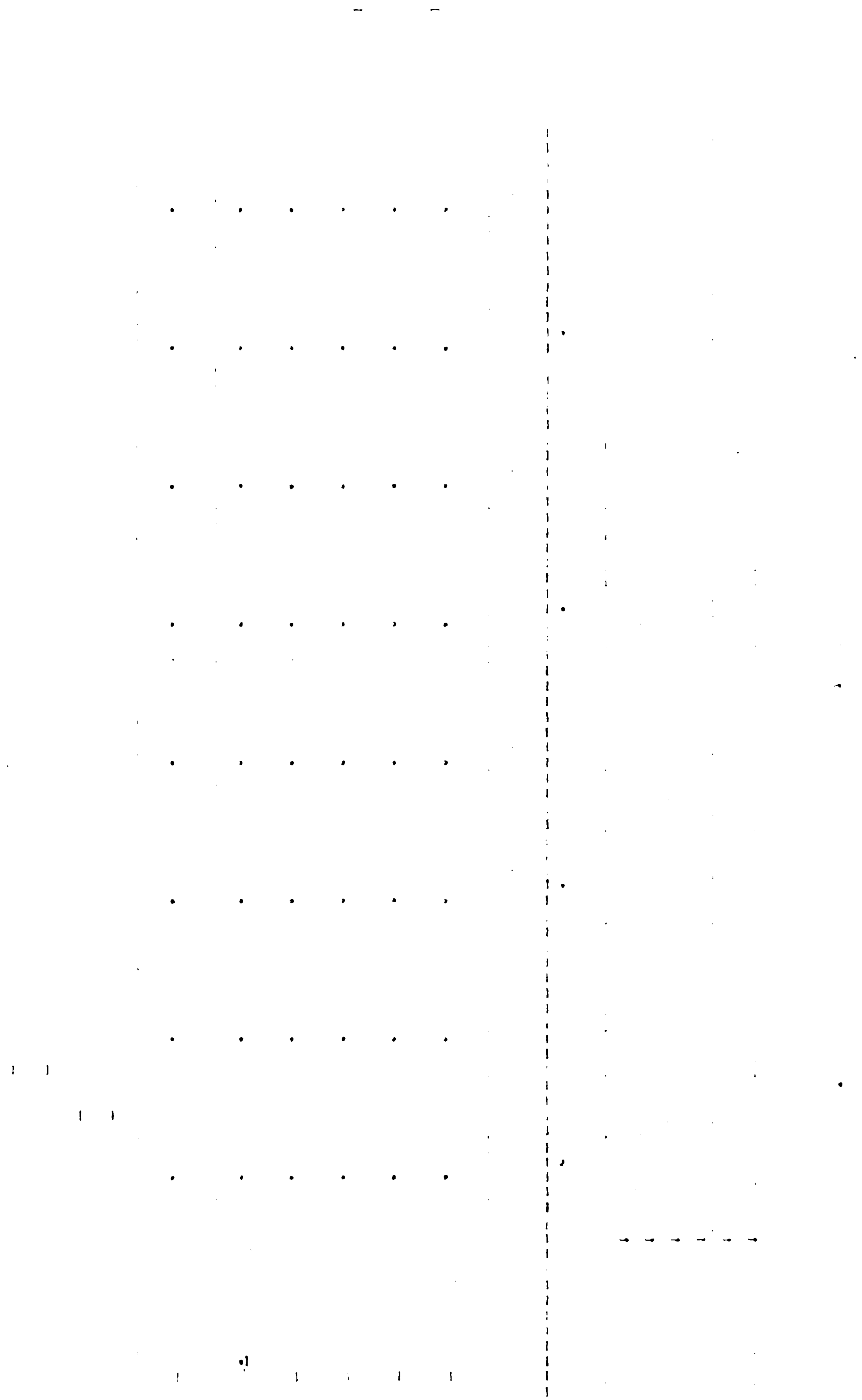
The amounts (percent and milligrams) of cellulose degraded when alfalfa and oat straw holocellulose were fermented are presented in Table 8. There were no significant differences in the extent of cellulose degradation due to treatment.

As with the cellulose of whole alfalfa hay and oat straw there is an indication that oat straw cellulose was more resistant to degradation early in the fermentation than alfalfa cellulose. Only 2.7% of the cellulose on the OHO treatment and none of the cellulose on the AHO treatment was degraded during the first two hours of fermentation, while 7.5% of the alfalfa substrate on the AHA treatment and 8.6% of the cellulose was degraded on the OHA treatment. This would indicate that the resistance of oat straw cellulose to degradation observed early in the fermentation of the whole plant forages is not due to lignification since most of the lignin is removed during the preparation of holocellulose.

TABLE 8. CELLULOSE DISAPPEARANCE, HOLOCELLULOSE SUBSTRATE

| Period | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AHO[1] | | AHA[2] | | OHO[3] | | OHA[4] | |
| | Wt(mg) | | Wt(mg) | | Wt(mg) | | Wt(mg) | |
| 0 Hour | 129.2 | | 107.6 | | 175.2 | | 121.2 | |
| | Wt Dig | % Dig | Wt Dig | % Dig | Wt Dig | % Dig | Wt Dig | % Dig |
| 0-2 Hour | 00.0 | 00.0 | 8.4 | 7.5 | 4.7 | 2.7 | 11.9 | 8.6 |
| 2-4 Hour | 5.9 | 4.6 | 6.8 | 6.4 | 11.0 | 6.2 | 1.2 | 2.3 |
| 4-8 Hour | 24.4 | 18.3 | 12.8 | 11.9 | 32.5 | 17.9 | 19.7 | 17.1 |
| 8-12 Hour | 28.3 | 21.5 | 11.0 | 10.8 | 52.5 | 20.0 | 23.1 | 19.6 |
| 12-24 Hour | 37.2 | 28.8 | 24.8 | 25.8 | 71.6 | 42.5 | 32.9 | 28.3 |
| 0-24 Hour | 95.8 | 73.2 | 63.8 | 62.4 | 154.4 | 89.3 | 88.8 | 75.9 |

[1] Alfalfa innoculum-oat straw holocellulose
[2] Alfalfa innoculum-alfalfa holocellulose
[3] Oat straw innoculum - oat straw holocellulose
[4] Oat straw innoculum - alfalfa holocellulose



Oat straw innoculum tended to degrade more of the cellulose over the entire fermentation period than alfalfa innoculum. After four hours of fermentation all treatments had begun to degrade the cellulose extensively so that the extent of cellulose degradation was greater in every case than when the whole forages were fermented.

## <u>The production of volatile fatty acids from the cellulose of alfalfa hay and oat straw</u>:

The volatile acid production during each fermentation period from the fermentation of the cellulose of alfalfa hay and oat straw is summarized in Table 9. There were no significant differences in the total volatile acid production nor in the ratios of acetic to propionic acid due to treatment.

The volatile acid production tended to be greater when oat straw cellulose was fermented. However, this difference may have been due to the greater amount of cellulose present in these flasks. Treatment OCO (oat straw innoculum-oat straw cellulose) contained 178.7 mg. and ACO 175.4 mg. of cellulose while treatment OCA (oat straw innoculum-alfalfa substrate) contained 75.6 mg. and treatment ACA (alfalfa innoculum-alfalfa substrate) 73.3 mg. of cellulose. The discrepancy in the amounts of substrate available for fermentation in these treatments and the greater amounts of volatile acids produced on treatments which contained greater amounts of cellulose lead to the conclusion that the quantity of cellulose in the treatments containing alfalfa cellulose limited the volatile acid production.

## TABLE 9. VOLATILE FATTY ACID PRODUCTION DURING THE FERMENTATION OF ALFALFA AND OAT STRAW CELLULOSE BY FERMENTATION PERIOD

| Fermentation Period | Fraction[1] | Treatment[4] |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|
|  |  | OCO |  | OCA |  | ACA |  | ACO |  |
|  |  | Amt.[2] | Ratio[3] | Amt. | Ratio | Amt. | Ratio | Amt. | Ratio |
| 0-2 Hrs | 1 | 18 |  | 18 |  | 58 |  | 00 |  |
|  | 2 | 12 | 1.50 | 28 | 3.79 | 18 | 8.56 | 00 | 0.00 |
|  | 3 | 18 |  | 106 |  | 154 |  | 00 |  |
|  | Total | 48 |  | 152 |  | 230 |  | 00 |  |
| 2-4 Hrs | 1 | 26 |  | 16 |  | 20 |  | 42 |  |
|  | 2 | 48 | 2.83 | 28 | 2.64 | 36 | 2.00 | 16 | 6.25 |
|  | 3 | 136 |  | 74 |  | 72 |  | 100 |  |
|  | Total | 210 |  | 118 |  | 128 |  | 158 |  |
| 4-8 Hrs | 1 | 14 |  | 30 |  | 08 |  | 68 |  |
|  | 2 | 52 | 1.31 | 48 | 2.46 | 24 | 2.83 | 64 | 3.03 |
|  | 3 | 68 |  | 118 |  | 68 |  | 194 |  |
|  | Total | 134 |  | 196 |  | 100 |  | 326 |  |
| 8-12 Hrs | 1 | 38 |  | 28 |  | 46 |  | 04 |  |
|  | 2 | 30 | 2.07 | 22 | 3.27 | 44 | 2.55 | 64 | 0.25 |
|  | 3 | 62 |  | 72 |  | 112 |  | 26 |  |
|  | Total | 130 |  | 122 |  | 202 |  | 94 |  |
| 12-24 Hrs | 1 | 74 |  | 52 |  | 184 |  | 140 |  |
|  | 2 | 380 | 1.88 | 194 | 1.62 | 132 | 2.55 | 450 | 1.86 |
|  | 3 | 714 |  | 314 |  | 336 |  | 836 |  |
|  | Total | 1168 |  | 560 |  | 652 |  | 1426 |  |
| 0-24 Hrs | 1 | 172 |  | 144 |  | 316 |  | 254 |  |
|  | 2 | 522 | 1.91 | 320 | 2.14 | 254 | 2.92 | 594 | 1.95 |
|  | 3 | 998 |  | 684 |  | 742 |  | 1156 |  |
|  | Total | 1692 |  | 1148 |  | 1312 |  | 2004 |  |

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[1] Fraction 1 = Butyric + higher acids; 2 = propionic acid; 3 = acetic acid.

[2] Expressed as microequivalents of acid

[3] Ratio of acetic/propionic

The ratio of acetic to propionic acid tended to be higher when alfalfa cellulose was the substrate. As with holocellulose this trend is opposite to that observed with the whole plant material. These observations would lead to the conclusion that the cellulose and holocellulose of alfalfa hay are not responsible for the tendency of alfalfa hay to produce a lower ratio of acetic to propionic acid. The fact that the lower ratio observed when the fermentation of alfalfa hay was compared to that of oat straw was significantly lower only during the first four hours of fermentation would support this conclusion since little holocellulose appears to be degraded during the early part of the fermentation.

### Cellulose disappearance on the fermentation of the cellulose of alfalfa hay and oat straw:

The amounts (percent and milligrams) of cellulose degraded when alfalfa and oat straw cellulose were fermented are presented in Table 10. There were no significant differences in the percents of cellulose degraded due to treatment.

The treatments which contained oat straw cellulose (OCO and ACO) degraded more cellulose than those containing alfalfa cellulose (ACA and OCA). However, there were considerably greater amounts of cellulose present in the treatments containing oat straw cellulose. The differences in amounts of cellulose degraded were probably influenced by the amounts available for fermentation.

In the case of the percents of cellulose degraded it is apparent that oat straw innoculum tended to degrade cellulose

TABLE 10. CELLULOSE DISAPPEARANCE ON THE FERMENTATION OF ALFALFA AND OAT STRAW CELLULOSE

| Period | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | OCO[1] | | OCA[2] | | ACA[3] | | ACO[4] | |
| | Wt (mg) | | Wt (mg) | | Wt (mg) | | Wt (mg) | |
| 0 Hour | 178.7 | | 75.6 | | 73.3 | | 175.4 | |
| | Wt Dig | % Dig | Wt Dig | % Dig | Wt Dig | % Dig | Wt Dig | % Dig |
| 0-2 Hr. | .8 | .04 | .0 | .00 | .0 | .00 | 1.1 | .60 |
| 2-4 Hr. | 11.1 | 6.0 | 6.7 | 8.40 | 2.2 | 3.1 | 5.3 | 3.20 |
| 4-8 Hr. | 1.4 | .9 | 2.4 | 3.30 | 13.9 | 19.0 | 11.3 | 6.30 |
| 8-12 Hr. | 9.1 | 5.4 | 4.0 | 5.81 | 11.9 | 14.8 | 23.7 | 13.50 |
| 12-24 Hr. | 81.7 | 46.6 | 13.0 | 26.10 | 28.3 | 38.1 | 93.2 | 53.10 |
| 0-24 Hr. | 104.1 | 59.3 | 31.1 | 43.61 | 56.3 | 75.0 | 134.6 | 76.7 |

[1] Oat straw innoculum-oat straw cellulose

[2] Oat straw innoculum-alfalfa cellulose

[3] Alfalfa innoculum-alfalfa cellulose

[4] Alfalfa innoculum-oat straw cellulose

less completely than alfalfa innoculum. Treatment OCO degraded 59.3% while treatment OCA degraded 43.6% of the cellulose present as compared to 76.7% and 75.0% for treatments ACA and ACO respectively.

All treatments attacked the cellulose rather slowly with negligible amounts of cellulose being degraded during the first two hours of fermentation. Alfalfa cellulose appeared to be more readily attacked than oat straw cellulose. After 8 hours of fermentation treatment ACA had degraded 21.1% and treatment OCA 11.7% of the cellulose present as compared to 6.9% and 9.5% for treatments OCO and ACA respectively. These figures also show that alfalfa innoculum was more active cellulolytically early in the fermentation. Oat straw innoculum appeared to be relatively inactive through the first 12 hours of fermentation. Most of the cellulose degradation took place during the last 12 hours. The low values observed for total cellulose degradation by oat straw innoculum suggest that this particular batch may have been inferior to that used in the fermentation of holocellulose where the extent of degradation of cellulose with oat straw innoculum was much greater.

## The fermentation of hemicellulose:

This experiment contained only two treatments since the preparation of hemicellulose yielded small amounts and not enough was collected for the usual 2 x 2 factorial experiment. Alfalfa innoculum was used to ferment both alfalfa and oat straw hemicellulose. The results of this experiment are summarized in Table 11.

TABLE 11. VOLATILE FATTY ACID PRODUCTION BY PERIODS FROM THE IN VITRO FERMENTATION OF ALFALFA AND OAT STRAW HEMICELLULOSE BY ALFALFA INNOCULUM.

| Fermentation Period | Fraction[1] | Treatment | | | |
|---|---|---|---|---|---|
| | | AXA | | AXO | |
| | | Amt.[2] | Ratio[3] | Amt. | Ratio |
| 0-2 Hrs. | 1 | 40 | | 28 | |
| | 2 | 82 | 2.34 | 62 | 2.19 |
| | 3 | 192 | | 136 | |
| | Total | 314 | | 226 | |
| 2-4 Hrs. | 1 | 22 | | 20 | |
| | 2 | 68 | 1.94 | 104 | 2.12 |
| | 3 | 132 | | 220 | |
| | Total | 222 | | 344 | |
| 4-8 Hrs. | 1 | 60 | | 54 | |
| | 2 | 54 | 2.56 | 52 | 2.19 |
| | 3 | 138 | | 114 | |
| | Total | 252 | | 220 | |
| 8-12 Hrs. | 1 | 42 | | 20 | |
| | 2 | 00 | --- | 70 | 2.29 |
| | 3 | 34 | | 160 | |
| | Total | 76 | | 250 | |
| 12-24 Hrs. | 1 | 78 | | 114 | |
| | 2 | 158 | .91 | 158 | 1.32 |
| | 3 | 144 | | 208 | |
| | Total | 380 | | 480 | |
| 0-24 Hrs. | 1 | 242 | | 236 | |
| | 2 | 358 | 1.79 | 450 | 1.86 |
| | 3 | 640 | | 838 | |
| | Total | 1240 | | 1524 | |

---

[1] Fraction 1 = butyric plus higher acids; 2 = propionic acid; 3 = acetic acid.

[2] Microequivalents acid.

[3] Ratio acetic/propionic acid.

The apparent preference of innocula for familiar substrate is reflected in this fermentation as it was in the case of all other experiments. The alfalfa innoculum alfalfa substrate treatment (AXA) produced a greater amount of volatile fatty acids during the first two hours of fermentation than the alfalfa innoculum-oat straw hemicellulose combination (AXO). This lag in fermentation indicates that there may be basic structural differences in the hemicellulose of alfalfa and oat straw not related to lignin since essentially all of the lignin was removed during the preparation of hemicellulose.

From the 2nd to the 4th hour the AXO production of volatile acids surpassed that of the AXA treatment. There was slightly more total acids produced on the AXO treatment for the entire fermentation period. The ratios of acetic to propionic acids produced on these two treatments did not differ greatly.

# DISCUSSION

## Cellulose Degradation of the Whole Plant and Fractions of the Plant

The percents of total cellulose degraded for the 24 hour fermentation period on the various treatments are summarized in Table 12.

TABLE 12. PERCENT CELLULOSE DEGRADED DURING THE 24 HOURS OF FERMENTATION

| Substrate | Treatment | | | |
|---|---|---|---|---|
| | 00 | 0A | A0 | AA |
| Whole Plant | 40.3 | 47.4 | 38.8 | 37.8 |
| Holocellulose | 89.3 | 75.9 | 73.2 | 62.4 |
| Cellulose | 59.3 | 43.6 | 76.7 | 75.0 |

In all cases the purified products were degraded to a greater extent than the natural materials, except for the oat straw innoculum-alfalfa cellulose treatment. This is a treatment which had a low cellulose concentration in the fermentation flask and apparently a relatively inactive innoculum. It is possible that this value does not represent the normal case. This reasoning is supported further by the fact that oat straw innoculum, which was obtained on a different day, degraded the cellulose of alfalfa holocellulose further than cellulose contained in the whole forage.

Analysis of variance of the cellulose degradation of whole forage cellulose versus the cellulose degradation of holocellulose and cellulose prepared from the forage indicates that a significantly ($p < .05$) greater percentage of cellulose was degraded in the plant fractions.

Salsbury *et al.* (1956) also obtained increased cellulose digestion when they compared the *in vitro* digestion of the intact plant with cellulose prepared from that plant.

The increased degradation observed in the plant separates may be attributed to several causes. In preparation of cellulose and holocellulose the treatment may partially degrade the cellulose making it more easily attacked by rumen microflora. Lignin is removed during the preparation of the holocellulose and cellulose. This may also have been a factor in the more complete degradation of the material.

The fermentation of holocellulose led to the production of a significantly lower ($p < .05$) ratio of acetic to propionic acid than either cellulose or intact forages. Since hemicellulose and cellulose make up holocellulose the inference is that hemicellulose fermentation would lead to the production of a low ratio of acetic to propionic acids. This was found to be the case when hemicellulose was fermented although the ratio was not less than that observed on the fermentation of holocellulose.

## The Importance of Cellulose to Volatile Fatty Acid Formation

Figure 2 is a visual projection of the microequivalents of volatile fatty acids produced per milligram of cellulose degraded for each treatment. This is an expression of the relative importance of the degradation of cellulose to the production of volatile fatty acids.

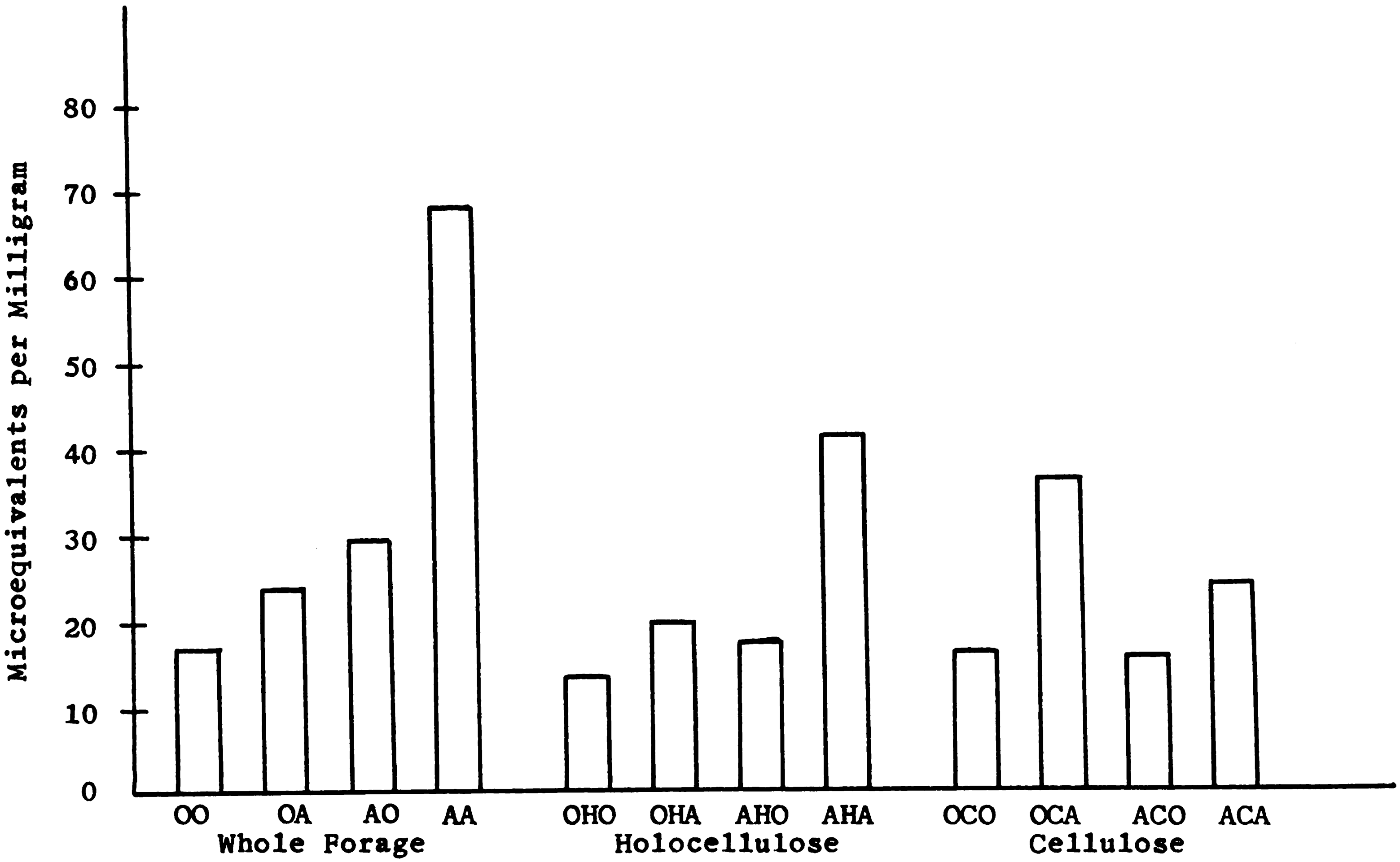

FIGURE 2. MICROEQUIVALENTS OF VFA PRODUCED PER MILLIGRAM OF CELLULOSE DEGRADED

The first four bars represent the relation between volatile acid production and cellulose degradation when the whole forages were fermented. The oat straw innoculum-oat straw substrate combination (OO) produced the fewest microequivalents of volatile fatty acids for each milligram of cellulose fermented followed by the oat straw innoculum-alfalfa substrate treatment (OA) and the alfalfa innoculum-oat straw substrate treatment with the alfalfa innoculum-alfalfa substrate treatment producing the greatest total amount of volatile fatty acids for each milligram of cellulose degraded.

Since there are undoubtedly materials other than cellulose which contribute to the formation of volatile fatty acids, the lower values are indicative of a greater importance of cellulose to the formation of volatile fatty acids. Therefore, the cellulose of oat straw was more important than that of alfalfa in the formation of volatile fatty acids. Oat straw innoculum also depended on the degradation of cellulose to a greater extent than alfalfa innoculum. These relationships suggest that alfalfa contains more materials other than cellulose which contribute to the formation of volatile acids.

It is also apparent that alfalfa innoculum must have produced more volatile acids from material carried over in the innoculum since it was able to produce more volatile acids per mg. of cellulose degraded than oat straw innoculum. The AA treatment produced more than twice the amount of volatile acids per milligram of cellulose degraded than any other treatment. The reason for this is not readily apparent since only a small increase in volatile acid production can be accounted for by the difference in substrate. A larger increase may be attributed to the source of innoculum.

An attempt was made to put a quantitative estimate on the effect of innoculum and substrate by subtracting the value for 00 from cross-over treatments. Thus 0A-00 = 7.52 microequivalents of additional microequivalents volatile acids produced per mg. cellulose degraded due to alfalfa substrate versus oat straw substrate and A0-00 = 13.05 microequivalents of additional volatile fatty acids produced due to innoculum. The sum of these effects equals 20.57 microequivalents of additional volatile fatty acids which can be attributed to alfalfa innoculum and alfalfa substrate if no other factors influenced the volatile acid production. The measured difference between treatments 00 and AA was 51.92 microequivalents. Thus the differences in volatile acid production due to source of innoculum and substrate can account for only about half of the actual measured difference between treatments AA and 00.

When the substrate was holocellulose a slightly different relationship was obtained. Treatment OHO (oat straw innoculum-oat straw holocellulose) continued to depend to a greater extent on the degradation of cellulose for the production of volatile fatty acids than any other treatment. The relative rank of the cross-over experiments was reversed with AHO being less than OHA. The alfalfa innoculum-alfalfa holocellulose treatment appeared to depend to the least extent on the degradation of cellulose for volatile acid production.

If the effect of substrate and innoculum are estimated as before, it is seen that OHA-OHO = 6.42 microequivalents more volatile fatty acids produced per milligram of cellulose degraded on the fermentation of alfalfa holocellulose versus oat straw holocellulose. There were 3.95 additional microequivalents of volatile fatty acids produced per milligram of cellulose degraded when alfalfa innoculum fermented oat straw holocellulose (OHO) as compared to oat straw innoculum fermenting oat straw holocellulose (AHO). The sum of these differences (10.37) represents the additional microequivalents of volatile acids produced per milligram of cellulose degraded due to the effect alfalfa innoculum versus oat straw innoculum and alfalfa holocellulose versus oat straw holocellulose. The measured difference between treatments OHO and AHA was 27.80 microequivalents of volatile fatty acids per milligram of cellulose degraded. Thus, differences in the holocellulose of alfalfa and oat straw and in alfalfa and oat straw innoculum can account for only 37% of the measured difference in microequivalents of volatile acids produced per milligram of cellulose degraded.

The relationships when oat straw and alfalfa cellulose was fermented are different from those observed on the fermentation of the whole forage and holocellulose. The oat straw innoculum-alfalfa cellulose treatment (OCA) produced the most microequivalents of volatile fatty acids per milligram of cellulose degraded followed by the alfalfa innoculum alfalfa cellulose treatment (ACA), the oat straw innoculum-oat straw cellulose treatment (OCO) and the alfalfa innoculum-oat straw cellulose treatment (ACO).

The results on the oat straw innoculum treatments (OCO and OCA) appear to be in error since the removal of all fermentable material except cellulose should lower the measured values for the microequivalents of volatile fatty acids produced per milligram of cellulose degraded. The oat straw innoculum used in this particular experiment appeared to be less active than innocula procured for previous experiments. There was a small amount of cellulose degraded and a relatively small amount of volatile acids produced particularly on the OCA treatment. This would tend to increase the values observed since volatile acids produced from material carried over in the innoculum would be relatively more important when small amounts of cellulose were degraded. The values for alfalfa innoculum treatments (ACO and ACA) decreased as was expected. It was also relatively more active cellulolytically than oat straw innoculum.

Because of the apparent discrepancy with oat straw innoculum, comparisons such as that made on previous experiments cannot be made. However, it appears from the relation on the ACA and ACO treatments that one milligram of alfalfa cellulose will produce more microequivalents of volatile fatty acids than one milligram of oat straw cellulose.

With the exception of the OCO and OCA treatments there was a decrease in the amount of volatile acids produced as the substrate was simplified.

Apparently, factors other than fermentable substrate and innoculum source caused the production of greater amounts of volatile fatty acids on the alfalfa innoculum-substrate treatment. This may be related to the greater susceptibility of alfalfa hay and alfalfa holocellulose and cellulose to bacterial attack. Because of this the *in vitro* fermentation was usually more active early in the fermentation period. This could have resulted in maintenance of a greater number and variety of microflora which were able to degrade cellulose with a greater efficiency of volatile fatty acid production.

### The Rate of Voluntary Consumption and Fermentation

The results of these experiments support the contention of Crampton (1957), McCullough (1959) and Blaxter (1956) that the worth of a forage depends on its voluntary consumption by animals. When the alfalfa hay and oat straw rations were fed *ad libitum* to cows during a digestion trial (Part I) the 4 cows consuming alfalfa hay ate an average of 23 pounds per head daily. The cows receiving the oat straw ration would eat an average of only 14 pounds of straw per day. One of these straw fed cows would not consume uniform amounts. The actual measured TDN values were not widely different being 64.0% for alfalfa hay and 61.6% for oat straw. Consequently, the voluntary consumption would be a limiting factor to the productive value of the oat straw.

The method of *in vitro* fermentation used in these experiments showed that alfalfa hay was fermented at a faster rate with the production of greater amounts of volatile fatty acids than oat straw. Crampton (1956) hypothesized that the rate of digestion and rate of passage of food through the digestive tract could be related to the voluntary consumption of the food. The recent work of Meyer *et al*. (1959) with pelleted rations seems to bear this out. These experiments show that alfalfa hay is fermented at a faster rate and consumed to a greater extent than oat straw.

## The Ratio of Acetic to Propionic Acid

The ratios of acetic to propionic acid produced on the *in vitro* fermentation of alfalfa hay and oat straw were not significantly different after a 24 hour fermentation period. This does not necessarily mean that different ratios do not occur on *in vivo* fermentation of alfalfa hay and oat straw.

When the ratios produced after 2, 4, 8, 12 and 24 hours of fermentation were compared, it was found that for the first four hours the fermentation of alfalfa hay resulted in the production of a significantly ($p < .05$) lower ratio of acetic to propionic acid. At the next and subsequent observation points no significant differences in ratio occurred.

It may be significant that the ratios on all treatments declined as the *in vitro* fermentation progressed. The same observation was made by Gray *et al*. (1952) who also noted that a similar decline did not occur *in vivo*.

Leffel (1958) stated that after 2 hours of *in vitro* fermentation the proportions of volatile fatty acids produced had changed from those observed *in vivo*. Warner (1956) determined that after 8 hours, *in vitro* fermentations conducted under the best of conditions were no longer representative of those expected *in vivo*. Maki and Foster (1957) experienced great difficulty in culturing acetic acid producing bacteria and concluded that they required strictly controlled conditions which were difficult to produce in the laboratory. Therefore, the tendency for *in vitro* rumen fermentations to produce lower amounts of acetic acid than expected on the basis of *in vivo* observations could be due to the reluctance of acetic acid bacteria to grow under laboratory conditions. The *in vitro* decline in acetic acid proportion may be characteristic of the fermentation system and not of the substrate being fermented.

From these considerations and from the results of the experiments reported herein it is considered to be entirely possible that under *in vivo* conditions there actually is a greater proportion of acetic acid produced on the fermentation of oat straw than on the fermentation of alfalfa hay. It may be concluded that the *in vitro* method of fermentation does not furnish accurate information about the relative proportions of volatile fatty acids produced on *in vivo* rumen fermentation.

## Source of Innoculum

Significant differences in the rate, extent and proportions of volatile acids produced occurred only when forages were fermented with innoculum obtained from animals which were maintained on that forage. This would indicate that the substrate fermented exerts its influence on the fermentation through a selection of the types of microflora in predominance.

Asplund et al. (1958) also observed the importance of source of innoculum on the production of volatile fatty acids. In contrast to the results reported here, innoculum from an alfalfa fed animal produced a significantly higher ratio of acetic to propionic acid as compared to innoculum from an oat straw fed animal after 24 hours of fermentation. They also obtained the greatest differences in volatile acid production when innocula fermented familiar substrates.

Other workers have noted variations in innocula obtained from animals fed different rations. Most were concerned with the comparison of microflora from concentrate fed ruminants as compared to that from animals receiving roughages (Gall et al., 1949; Burroughs et al., 1950; Hunt et al., 1952; Hungate et al., 1952). However, Bryant and Burkey (1953) reported that the flora from an oat straw fed cow was less complex than that from an alfalfa fed cow.

These experiments and those of Asplund et al. (1958) lead to the conclusion that important differences exist in the types of microflora in predominance and the fermentation produced by the microflora obtained from animals fed different kinds of forage. Thus, in vitro comparisons of various forages are most likely to show significant differences when the innoculum for in vitro fermentation is obtained from an animal which has been maintained on that particular forage so that the innoculum actually represents the forage.

## Holocellulose, Hemicellulose and Cellulose

The degradation of holocellulose, hemicellulose and cellulose as a part of the whole forage proceeded at about the same rate and to the same extent. This indicates that these components may be in close contact with each other in the plant and when their structure has become broken down they are utilized equally well by rumen microflora.

The content of hemicellulose in many forages is nearly as great as that of cellulose. Hansen *et al.* (1958) reported an average of 25.2% (range 12.1% to 39.9%) hemicellulose in common forages. The cellulose content in a similar group of forages ranged from 27.4 to 52.9% and averaged 32.6%. Therefore, the hemicellulose fraction could make a large contribution to the volatile acids produced on rumen fermentation of forages.

The ratios of volatile acids produced on the fermentation of the isolated plant fractions (cellulose, holocellulose and hemicellulose) were less than those produced when the whole forage was fermented. Barnett and Reid (1957a, 1957b) obtained similar results. They concluded from this that something other than cellulose or other complex carbohydrates was responsible for the high proportions of acetic acid produced *in vivo* on forages. Gray and Pilgrim (1952a, 1952b) concluded from similar observations that the discrepancy between *in vitro* and *in vivo* volatile fatty acid production was due to the preferential absorption of propionic acid from the rumen. Neither of these explanations have been entirely satisfactory.

The low ratios of volatile acid production on the fermentation of complex plant carbohydrates have been very difficult to resolve in view of the consistently high correlation of the proportion of acetic acid produced with the amount of these complex carbohydrates contained in forages (Phillipson, 1952; Johns, 1955; Jamieson, 1959; Balch and Rowland, 1957; Card and Shultz, 1953; Reid, 1957; Stoddard _et al._, 1949 and others). Perhaps Barnett and Reid (1957) have indicated the reason for these inconsistencies when they observed that the ratio of acetic to propionic acid produced depended, not so much upon the particular component considered but upon the ease of its fermentation, the more easily fermented materials resulting in the production of a lower proportion of acetic acid.

The procedures used in isolating complex carbohydrates from plant material could be responsible for the low ratio observed on the fermentation of these fractions. These procedures subject the material to drastic conditions which could conceivably alter the original structure considerably. As Simple an operation as grinding hay (Powell, 1939; Balch, 1952, 1959) has been observed to cause a lowering of the proportion of acetic acid produced on its rumen fermentation. Cooking or heating has also been shown to change the volatile acid production (Balch _et al._, 1955a; Shaw _et al._, 1959; Ensor _et al._, 1959).

Thus it is not inconceivable that the preparative procedures coupled with the characteristics of the _in vitro_ fermentation all combine to cause the _in vitro_ fermentation of purified

plant fractions to result in the formation of relatively greater proportions of propionic acid than fermentation as part of the whole forage *in vivo*, and that cellulose and hemicellulose as part of the whole forage could be important precursors of acetic acid in ruminants.

It was realized that these difficulties were inherent in the *in vitro* system of rumen fermentation before the investigation began; however, it was hoped that by using the comparative technique, differences in the volatile acid production on various feeds could be estimated. It was shown with this approach that the fermentation of alfalfa hay with innoculum obtained from an alfalfa fed steer as compared to the fermentation of oat straw with innoculum from an oat straw fed steer resulted in a significantly ($p < .05$) lower ratio of acetic to propionic acid only during the first four hours of fermentation, and that at the next time of observation after 8 hours of fermentation this difference was no longer significant. If there is a real difference in the proportions of volatile acids produced from alfalfa versus oat straw *in vivo*, these observations tend to agree with those of Warner (1956) who concluded that after 8 hours the *in vitro* fermentation no longer reflects the *in vivo* fermentation.

A technique such as that suggested by Stewart *et al.* (1958) which utilizes a series of short term *in vitro* fermentations to estimate the production of individual volatile fatty acids may be necessary for accurate prediction of *in vivo* volatile fatty acid production.

## SUMMARY

Oat straw and alfalfa-bromegrass hay were compared by conventional total digestible nutrient determinations, by *in vitro* volatile fatty acid production and cellulose digestibility.

The alfalfa-bromegrass hay was found to have an average of only 2.4% more TDN than oat straw. Four Holstein cows voluntarily consumed an average of 23 pounds of alfalfa-bromegrass hay per head daily; whereas, a similar group of 4 Holstein cows would consume an average of only 14 pounds of oat straw per head daily. On *in vitro* rumen fermentation alfalfa hay produced volatile fatty acids at a significantly ($p < .05$) faster rate than oat straw. The acetic to propionic acid ratio was ($p < .05$) significantly lower for alfalfa-bromegrass hay for the first four hours, but not significantly different for the balance of the 24 hour *in vitro* fermentation. The percent cellulose digestion on *in vitro* fermentation of alfalfa-bromegrass hay and oat straw was not significantly different.

Differences were significant only when innocula for the fermentations were obtained from steers fed the same forage as that being used for *in vitro* substrate.

The volatile fatty acid production (amount and ratio) and cellulose digestion of holocellulose and cellulose prepared from alfalfa-bromegrass hay and oat straw was not significantly different although early in the fermentation period cellulose digestion and volatile fatty acid production tended to be greater when innoculum fermented familiar substrate. Holocellulose and hemicellulose produced a significantly ($p < .05$) lower ratio of

acetic to propionic acid than whole forages or cellulose prepared from whole forages. The digestibility of the cellulose of holocellulose and cellulose prepared from the intact forage was significantly ($p<.05$) greater than the digestibility of cellulose in the intact forage.

Although the *in vitro* method utilized was able to distinguish between alfalfa hay and oat straw with respect to quantitative volatile fatty acid production the results on proportions of volatile fatty acids produced are considered inconclusive. The proportions of volatile fatty acids produced after 8 hours or longer of *in vitro* fermentation appear to be more characteristic of the fermentation system than of the substrate being fermented. A short term *in vitro* fermentation of less than 8 hours appears to be necessary for a differentiation between forages on the basis of proportions of volatile fatty acid.

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