IN VIVO AND IN VITRO EVALUATION OF THE NUTRITIVE VALUE OF FORAGES

by

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STATEMENT BY AUTHOR

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INTRODUCTION

The unique digestive system of ruminants enables them to utilize roughage as a major source of energy. Since it is generally one of the most economical constituents of the ruminant diet, it is important to obtain full use of it.

The two most important factors involved in the determination of the nutritive value of a forage are digestible energy concentration and voluntary intake per unit of animal body weight. Therefore, the feeding value of a forage is dependent upon the magnitude of its contribution to the daily energy requirements of the animal. Differences in forages depend largely upon the relative intake of them. Crampton (24) proposed that the extent of voluntary consumption of a forage is limited primarily by the rate of digestion of its cellulose and hemicellulose, rather than by contained nutrients or by the completeness of their utilization.

Since the utilization of the cellulose fraction of roughage is recognized as a primary factor in the nutrition of the ruminant, it has been proposed that the rate of *in vitro* cellulose digestion can be used as an indicator of cellulose digestion and ultimately as a predictor of *in vivo* roughage value.

Generally, feeding trials have been utilized with satisfactory results in evaluation of roughage; however, due to the time and cost involved in conducting experiments of this nature, a more expedient estimation of a particular forage value seemed desirable. The

in vitro fermentation technique has received much attention as a tool in the task of evaluation. *In vitro* fermentation is a term covering a variety of laboratory apparati, ranging from highly complex simulated rumen to the simplest of rumen microorganism inoculated flask incubations. Even the most elaborate "artificial rumen" technique can, at best, be used only as a screening device in studying influential factors in roughage from which the more promising results can ultimately be checked in animal experimentation. Several investigators, Donefer *et al* (31,32), Lloyd *et al* (56), and Crampton *et al* (25), have proposed the use of both *in vivo* and *in vitro* forage evaluation to determine the effectiveness of *in vitro* results in predicting *in vivo* response, or animal productivity.

This work was undertaken with use of *in vivo* methods to measure the value of three forages known to differ in feeding value (alfalfa, coastal bermuda and blue panic). *In vitro* cellulose fermentation curves of the three forages were then established and the value of the *in vitro* data as predictors of the feeding value of the forages were evaluated to substantiate relationships already proposed, or to establish new relationships.

REVIEW OF LITERATURE

The nutritive value of forages has been studied under a great variety of techniques. Within recent years many new approaches have been utilized with varying degrees of success. Likewise, various fractions of forages have been investigated regarding replacement value in the ration or as the sole source of meeting nutritional requirements of animals.

Crampton and Maynard (26) presented data indicating limitations of crude fiber and nitrogen-free extract estimates in determining nutritive value. They found the estimation of lignin, cellulose, and other carbohydrate fractions to be of more value than crude fiber and nitrogen-free extract determinations in predicting nutritional value of roughage. However, Walker and Hepburn (84) found no increase in the accuracy of predicting gross digestible energy by estimating lignin and cellulose fractions rather than crude fiber alone with the hays. Sullivan (78) found digestibility coefficients of cellulose to range from 56 - 89 percent, a range as great although not equal to crude fiber values. Lignin was found to be up to 10 percent digestible. Meyer and Jones (65) report lignin, crude fiber, and crude protein to be good indications of feeding value of alfalfa as an energy source. Stallcup (75) reported lignin content of legumes to be greater than in grass hays; however, he recognized the crude fiber was quite variable depending on stage of growth when harvested. Further

investigation also indicated that grass hays were generally higher in cellulose than alfalfa hay.

Burroughs et al (19) showed that variations in roughage qualities were attributable to the nutrients available for support of microorganisms of the rumen. Furthermore, Swift (81) suggests energy and protein to be two principal items of interest when evaluating forages. Swift (81) also reports digestible energy to be the best nutritive yardstick in comparing value because of speed and accuracy of determination.

Comparisons of coastal bermuda and alfalfa hays have been made by King et al (53) showing coastal bermuda, if properly fertilized, rapidly grown, cut at correct stage, and properly cured, was equal in protein content and worth 87 percent as much as alfalfa in feeding value. Alexander et al (1) also emphasized need for nitrogen fertilization to obtain hay with desirable crude protein content. Hammes et al (42) found coastal bermuda to be lower in protein and ether extract but higher in crude fiber and nitrogen-free extract than alfalfa hay. He also observed lower output per animal as a result of decreased dry matter consumption rather than a reduced coefficient of digestion. Suman et al (80) obtained rather desirable gains on steers with coastal bermuda grass; however, the carcass quality was inferior to that of the dry lot fed group. Beardsley et al (11) found steers consumed 2.1 pounds of chopped coastal bermuda per 100 pounds of body weight and gained 1.39 pounds per head daily.

Several attempts have been made to increase feed intake, gains,

and feed efficiency of coastal bermuda hay. Berry (13) observed dramatic results by pelleting the low quality roughages. He reported fine grinding and pelleting practically eliminated cud chewing, hastened movement of feedstuffs along the digestive tract, and usually brought about lower total energy loss with increases in propionate production within the rumen which is associated with more efficient gains.

Selectivity on the part of an animal has been studied. Williams and Evans (86) observed that sheep selected the less fibrous material of higher protein content when fed bracken hay. Grinding and pelleting of forages have been shown to solve this problem of selectivity.

Other principal factors that have been studied which account for nutritive value differences are digestible energy and voluntary intake of forage. Factors affecting intake are, according to Campling et al (21,22), reticulo-ruminal contents, or rate of disappearance from the reticulo-rumen. McCullough (63) states that consumption is also limited by mechanical satiation by bulk; however, he recognized the influence of palatability upon voluntary consumption. Blaxter et al (15) hypothesized appetite for food would fail at a particular distention of the alimentary tract of which dry matter content or fill is an index. These investigators further observed that increasing the feeding level resulted in an increased rate of food passage and decreased digestibility. Therefore, Blaxter proposed rate of passage through the gut to be prediction of digestibility of that food. Appetite was also correlated with food quality.

Crasemann (27) believed satiety of nutrients and energy to be

a more important factor in voluntary intake than ballast. However, Crampton (24) hypothesized that digestion of cellulose and hemicellulose rather than contained nutrients limit voluntary consumption of forage. Stallcup et al (76) also observed the influential effect of lignin on digestion by slowing up of passage of nutrients through the rumen, thus reducing physical capacity of animal for more roughage. Balch (7) found that the depression in digestibility of crude fiber in the reticulo-rumen was not a result of a change in rate of passage but suggested that it may be attributed to changes in fluidity of digesta of the reticulo-rumen. He found that the breakdown of crude fiber in the reticulo-rumen was more rapid when ruminal contents were more fluid.

Crampton (24) concludes that the rate of digestion may be retarded by any one of a number of circumstances reducing activity of rumen microflora such as excess lignification, deficiency of nitrogen or other specific minerals, or presence of excessive bacteriostatic agents.

To increase consumption and rate of passage, Gardner et al (37) compared long, chopped, ground, ground and pelleted hay with young calves. They found daily ad libitum consumption of hays progressively increased in order listed. Later, King et al (54) reported pelleted hay passed through the digestive tract almost twice as fast as loose hay. Alexander et al (2) reported that crude fiber digestion was significantly lower in ground and pelleted hay as compared to long hay.

O'Dell et al (66) suggests that rapid passage of ground and pelleted hays would appear to explain the decrease in digestibility of these forms. Lloyd et al (56) also observed a progressive decrease in energy digestibility in going from chopped to ground to pelleted forages, but found this was more than compensated for by progressively greater increase in relative intake.

Various in vitro procedures and apparati for evaluating forages have been used. Gall et al (35,36), Bryant (16), Huhtanen et al (48), Doetsch et al (30) have reported investigations concerning microbial populations. Other reviews have been written by Marston (60), Goss (38), McAnally and Phillipson (62), and Owen (67), to mention just a few. Burroughs et al (19) list these advantages of artificial rumen over in vivo trials: Speed of determination, greater control exercised over conditions, and reduced expense of experimentation. Of course there are enumerable limitations, if not in the procedures themselves, in the inferences that can be drawn on the rumen itself. Refinements of the in vitro techniques have been studied by Petersen (68) by use of pure cultures of rumen microorganisms. Hueter et al (47) acknowledges the use of the method; however, he believes results should be interpreted with caution until verified by in vivo experimentation. Cheng et al (23) used the method of "washed suspension" of rumen microorganisms which is a very sensitive technique. Hubbert et al (45,46) carried out intensive studies on mineral requirements of rumen microorganisms for cellulose digestion by this method. Huhtanen et al (48) used a minature artificial rumen. Pigden et al

(70) used an *in vitro* fermentation procedure. Warner *et al* (85) utilized this method and extended it by use of a dialysing sac with complex mineral mixture. Baumgardt *et al* (10) also worked with this general method, McDougall's artificial sheep saliva, and strained rumen fluid. Others, Taylor *et al* (83), used raw sheep rumen liquor. Other modifications have been made in collection of inoculum as reported by Johnson *et al* (51). They developed a so-called improved inoculum for *in vitro* fermentation by discarding the first extraction of the rumen fluid and resuspending the pressed pulp in buffer; the liquid in turn being centrifuged and rumen microorganisms resuspended in a mineral solution as the prepared inoculum. Rumen inoculum prepared this way gave higher cellulose digestion and less variation between experiments.

Authors cited on variables associated with different *in vitro* procedures are, first of all, those that have studied substrate levels, size, source, method of preparation of inoculum. Cheng *et al* (23) obtained most favorable digestion of cellulose when it consisted of 1 percent or less of the medium. Taylor *et al* (83) used .3 gram of hay with 20 ml raw rumen liquor and 7.5 ml artificial saliva. Baumgardt *et al* (10) used 1 gram sample with 25 ml rumen fluid plus McDougall's artificial saliva. Despite the variations in the substrate levels used, Kamstra and Quicke found nearly the same percentage cellulose digestion for levels from .44 - 2.50 grams which represent cellulose levels of .08 - .48. Regarding inoculum size, MacLeod and Brumwell (58) found that with large amounts of inoculum, supplemental additions of nutrients produced little additional responses. However, Lloyd *et al* (56)

observed that the particle size of the substrate influenced cellulose digestion. Later, Dehority and Johnson (29) found ball milling increased the quantity of cellulose digested and that the increase became larger with advancing maturity and lignification of the forage. Warner (85) showed that for a microbial population to remain normal in numbers and activity, it was necessary to use as the in vitro test substrate only substances similar to those in the diet fed the animal from which the inoculum was taken. However, Quicke et al (72) reported no effect on in vitro cellulose digestion with use of steers fed on different forages. Also, similar in vitro cellulose digestive coefficients were observed by Le Fevre et al (55) on sheep or cattle rumen fluid. Later Quicke et al (72) found little difference in cellulose digestion as measured by using strained rumen juice, phosphate buffer extract of pressed rumen contents, or resuspended ruminal microorganisms. Upon transferring inoculum as many precautions as possible should be taken to approach ideal situation. Louw et al (57) recommends the least delay, exposure to air, and loss of heat as is physically possible. Other variables that must be considered to obtain optimum cellulose digestion consist of pH, carbon dioxide, temperature, and sample agitation. Cheng et al (23) suggested use of carbon dioxide to provide anaerobic conditions as well as agitate the solution. However, Marston et al (61) found too rapid of stirring caused depression in fermentation in vitro. Nitrogen and carbon dioxide was utilized by Warner (85). Thirty-nine degrees Centigrade seems to be the temperature used most frequently during incubation as

reported by Warner (85), Cheng et al (23) and Baumgardt et al (10). The above mentioned authors also found a pH range of 6.8 - 7.6 to be optimum for cellulose digestion. However, they failed to find any serious reduction in cellulose digestion unless pH dropped below 6.4.

Various incubation lengths have been used depending on the kind of in vitro procedure utilized. Baumgardt et al (10) found no advantage in extending the fermentation period beyond 24 hours for digestible energy estimates. Dehority and Johnson (29) found almost complete utilization of available cellulose at the end of 30 hours incubation. Burroughs et al (18) used slightly longer fermentation periods to allow for the inferior in vitro starting conditions. With 24 hour periods Cheng et al (23) obtained 60 percent cellulose digestion with a 5 percent cellulose solution. Susceptibility of purified cellulose to attack is influenced by source and method of preparation as reported by Baker et al (6). It has been observed by Louw et al (57) that microorganisms attack cellulose at broken or torn ends. There was no correlation between degree of polymerization and rate of in vitro digestion of cellulose as determined by Baker et al (6). His experimental evidence, however, suggests a greater proportion of amorphous material (as contrast to crystalline regularity) in hay cellulose than purified cellulose.

The rates of in vitro cellulose digestion for alfalfa and grasses has been compared by Baumgardt et al (10) and Donefer et al (31). Both found initial rates of alfalfa digestion to be greater and maximum obtained sooner than with grasses. The apparent maximum

digestibility of grass cellulose had not been reached yet at 48 hours. Dehority and Johnson (29) reported legume grass differences in regard to amount of cellulose digestion per given amount of lignin.

Microbial metabolism in the rumen depends largely on a high concentration of microorganisms, mainly bacteria and protozoa as reported by Peterson (68). Nutritional constituents affecting these bacteria, protozoa, yeasts, and fungi also indirectly influence cellulose digestion. The effect of certain feeds, such as dried distillers solubles, ash of alfalfa extract, soybean oil meal, and linseed oil meal, were most helpful under experimental conditions imposed by Burroughs et al (20). Cane molasses, corn, wheaten bran, and cottonseed meal gave some response. Hall et al (40) suggested presence of unidentified factors in addition to B-vitamins responsible for favorable effect that yeast extract shows on cellulose digestion. Other feeds such as beef liver extract and whale solubles have influential effects as shown by MacLeod et al (58); later Hall et al (41) and others have demonstrated the inhibiting effects nitrates and nitrites play on in vivo and in vitro cellulose digestion. It has been shown many times that the addition of starch may stimulate synthesis of B-vitamins and increase nitrogen utilization, although with additions above a certain level, crude fiber digestibility is usually depressed as shown by Hunt et al (49). Le Fevre and Kamstra (55), Swift et al (82), Arios et al (4), and Head (43) have all observed this effect of concentrate feed (starch) upon cellulose digestibility. Burroughs et al (20) found that cellulose depression could be counteracted by addition of casein to the diet. In 1954, MacLeod and Brumwell (58) added a mixture of

amino acids, nitrogen, and ammonia as urea and observed them to be equally effective in stimulating cellulose digestion. The mineral requirements have been studied rather extensively by Burroughs et al (20), Hunt et al (49), Salsbury et al (74) and more recently by Hubbert et al (45,46) and Bryant et al (17). Hubbert et al (46) mentions sulfur, magnesium, and calcium to be inorganic elements most likely to be deficient for optimum cellulose digestion. However, phosphate, ammonium, and potassium ions have been demonstrated to be essential in vitro. Different investigators, Hall et al (40), Bentley et al (12), and Bryant et al (17) have demonstrated stimulating effect of B-vitamins, particularly biotin and PABA, on cellulose digestion.

Bentley et al (12) reported volatile fatty acids, primarily valeric acid, and to some extent butyric acid, to possess cellulolytic activity. The addition of high levels of fat by Grainger et al (39) and others have exhibited marked reduction in cellulose digestion; however, the inclusion of calcium or iron in the ration partially alleviates this effect in vivo.

A depressing effect of lignin on cellulose digestion in vitro has been shown by Stallcup et al (76), Kamstra et al (52), Quicke (71), and Dehority and Johnson (29). Kamstra et al (52) and Sullivan and Hershberger (79) found by separating cellulose from lignin, they obtained greatly improved digestion in vitro. Dehority and Johnson (29) present evidence that suggests the theory of lignin in forages acting as a physical barrier between cellulose and cellulolytic rumen bacteria, thereby influencing digestibility of forage. Stallcup (76) suggests

a second effect of lignin in that it slows up passage of nutrients through the rumen because there is more ingesta remaining in the rumen 12 hours after feeding, thus reducing the physical capacity of the animal for more roughage at the next feeding.

Other influential non-nutritional factors affecting cellulose digestion are length of time spent in alimentary tract as indicated above and effect of position in rumen as related to fluidity of rumen contents. Phillipson (69) found length of time in alimentary tract and particularly the rumen to influence the quantity of cellulose disappearing as a result of bacterial fermentation. Balch (7) reported that the loss in weight of cotton threads suspended in rumen of cow was invariably more rapid in ventral than dorsal portion possibly because the ventral contents are more fluid or more anaerobic.

In vitro measurements of forage quality are of little value unless they can be used to predict the in vivo value of roughage, except possibly for studying specific reactions that are of academic interest. However, many in vitro measurements have shown a reasonable degree of correlation with in vivo values. Barnett (8) found digestion coefficient of crude fiber in vivo and in vitro for silage cellulose significantly correlated. Asplund (5) reported high correlations between dry matter digestion coefficients in vivo and dry matter loss and volatile fatty acid production in vitro. Quicke et al (71), using inoculum from steer and digestibility trials run with sheep, found no significant difference between results obtained in vitro and in vivo with grass hays, but in some of the legume hays, cellulose digestibilities were significantly different. A

cuprietheylene diamine method developed by Dehority and Johnson (28) has shown the same results. Correlation coefficients of .97 and greater between cellulose digestion *in vitro* and *in vivo* have been reported by Hershberger *et al* (44) and Baumgardt *et al* (10). Le Fevre *et al* (55) found that 48 hour fermentations yielded cellulose digestion coefficients similar to those values obtained *in vivo*. Reid *et al* (73) reported 24 hour and 48 hour *in vitro* cellulose digestion highly correlated with *in vivo* dry matter digestibility. Although no consistent relationship existed between voluntary intake and rate of cellulose fermentation, percent cellulose digestion was significantly correlated with total digestible nutrients, digestible dry matter, digestible energy (Calories per gram), and energy digestive coefficients *in vivo* by Baumgardt *et al* (9,10). Recently Crampton *et al* (25), Lloyd *et al* (56), and Donefer *et al* (31,32) have proposed certain calculations from estimable values *in vitro*. A Nutritive Value Index (NVI) may be calculated from relative intake and energy digestibility data obtained *in vivo*. The relative intake (RI) value being calculated by formula is

$$RI = \frac{\text{Observed Intake} \times 100}{80\ (W_{kg}^{.75})}$$

Relative intake is expressed as percentage of normal intake by growing normally fleshed 60 - 95 pound sheep and the standard taken as 80 grams or .18 pounds per unit of metabolic size. Eighty grams (± 10 grams) was intake of early bloom, chopped dehydrated legume forage per unit of weight in kilograms raised to .75 power. The NVI obtained in

this way was shown to be highly correlated with 12 hour *in vitro* cellulose digestion, 12 hour x 24 hour *in vitro* cellulose digestion (*in vitro* index), and multiple correlated 12 and 24 hour values. It was observed that 12 hour and 24 hour *in vitro* cellulose digestion were significantly correlated with relative intake and digestible energy, respectively. They also proposed use of 12 hour *in vitro* cellulose digestion (X) in estimating NVI of forage (Y) by equation: $Y = -7.8 + 1.314X$. Later work indicated $r = .91$ with 26 forages fed chopped, and $r = .87$ with 16 forages fed ground, by using NVI calculated from *in vivo* data and 12 hour *in vitro* cellulose digestion values.

EXPERIMENTAL PROCEDURE

I. Forages

The coastal bermuda (*Cynodon* spp.) and blue panic (*Panicum antidotals*) used in the trials were grown at the Soil Conservation Service Plant Materials Center at Tucson, Arizona, during 1960. The forages used were cut on June 22 and August 17 with a 3 inch and 9-12 inch stubble height remaining for the bermuda and blue panic, respectively. The hays were baled in the field and chopped prior to being fed. The alfalfa hay was a high quality, sun-cured product. The bermuda and panic hays were medium to low quality.

Approximately 4 percent rendering plant tallow was added to the hays just before entering the hay grinder to reduce dust. Preliminary studies with alfalfa indicated that the addition of this level of tallow had no significant influence on dry matter digestibility or feed intake.

II. Experimental Animals

Six approximately 100 pound wethers were used for the voluntary forage intake measurements. The hays studied were fed for a 10-day preliminary period followed by a 15-day feeding period in which daily hay consumption per pen was recorded. Four of these animals were subsequently placed in metabolism crates for further digestion studies.

Rumen microorganism inoculum for the *in vitro* fermentation

studies was obtained from a fistulated steer maintained on an all-ground alfalfa ration.

III. Laboratory Facilities

A rectangular stainless steel tank (10" x 50" and 9" deep), heater circulator, carbon dioxide tank, 50 ml centrifuge tubes fitted with carbon dioxide inlet glass tubes and condensors, continuous flow centrifuge, and other miscellaneous items were employed for the in vitro experiments.

IV. Digestion and Metabolism Studies

Observations were made on voluntary dry matter intake, energy digestibility, and nitrogen balance studies of forages. Digestibility and nitrogen balance data were collected with four wethers on each hay evaluated. The individual animals were confined to metabolism crates during a preliminary period long enough to adjust the animals to a constant daily feed intake (at least 10 days). Fecal and urine collections were then made during a 5-day period.

Proximate analyses were made as outlined by the A.O.A.C. (1955) and gross energy was determined with the adiabatic oxygen bomb calorimeter.

V. In Vitro Fermentation Study

It appeared that some of these techniques would not lend themselves to rate of cellulose degradative determinations as well as others. Therefore, after a consideration of the alternatives, the apparatus utilized here was a combination of various methods developed

with certain modifications to fit our laboratory facilities. Realizing it would be impossible to simulate the environmental condition in the rumen, the author found the following described procedure performed most satisfactorily under the existing conditions. The method was basically one as described by Crampton et al (25). Cheng et al (23) reported pH was initially adjusted to 7.0 with saturated Na_2CO_3, and with buffering capacity of the medium no further pH adjustments during the 48 hour incubation period were necessary. Centrifuge tubes (50 ml) were used in the water bath maintained at 39 degrees Centigrade with a two tube in a line arrangement. Preliminary uniformity studies showed no effects of sequential arrangement due to transfer of substrate or toxic gasses into the second tube. Carbon dioxide was used to provide anaerobiosis as well as to agitate the solution as suggested by Huhtanen et al (48), Cheng et al (23), and Warner (85). The tubes consisted of substrate and McDougall's artificial ruminant saliva plus other additives shown to support optimum cellulose digestion in vitro. The composition of the artificial saliva and additives are given in the following table. Each tube was incubated with 25 milliliters of artificial saliva medium, 30 milligrams of urea, 25 milligrams of casein hydrolysate, 5 micrograms of biotin, 12.5 micrograms of para-animo-benzoic acid, and 7.5 milligrams of valeric acid.

A. In vitro collection procedure - Method of preparing the bacterial inoculum was, as developed by Johnson et al (51), a so-called improved inoculum for in vitro fermentation by discarding the first extraction of rumen fluid and resuspending the pressed pulp in

TABLE 1

COMPOSITION OF ARTIFICIAL SALIVA MEDIUM

AND OTHER ADDITIVES PER LITER

Sodium Phosphate (monobasic)	4.8 gms.
Sodium Bicarbonate	4.8 gms.
Potassium Chloride	.7 gm.
Sodium Chloride	.7 gm.
Magnesium Sulfate	.2 gm.
Calcium Chloride	.07 gm.
Other Additives	
Urea	1.2 gms.
Casein Hydrolysate	1.0 gms.
Biotin	.2 mg.
Para-Amino-Benzoic Acid	.5 mg.
Valeric Acid	.3 gm.

buffer; this liquid in turn being centrifuged and the rumen microorganisms resuspended in a mineral solution as the inoculum source. Inoculum prepared in this way gave high cellulose digestion and less variation between experiments. The procedure used here was nearly identical to one described above with the exception that microorganisms, after being resuspended in the PO_4 buffer, were transported to the laboratory as rapidly as possible in preheated, pregassed thermos jug, and rumen microorganisms were then filtered through 6 layers of cheesecloth and collected by continuous flow centrifuge at 18,000 rpm. The microorganisms were resuspended in artificial saliva and incubated with the addition of CO_2 for 30 minutes before the mixed suspension

was used to inoculate the samples. Precautions were taken to insure a homogenious mixture during pipetting 25 ml of rumen inoculum into each tube.

B. Substrate level and preparation - Most workers agree particle size of forage affects, within limits, the rate of cellulose digestion in vitro. Lloyd et al (56) found grinding of grass more advantageous than legumes such as alfalfa. Dehority and Johnson (29) reported ball milling increased subsequent cellulose digestion in vitro and this increase became larger with advancing maturity and lignification of the forage. Grinding the forage through a laboratory wiley mill was found to increase the amount of cellulose digestion obtained here also. Therefore, all of the following samples were ground through a 40 mesh screen before in vitro digestion. The substrate concentration in the medium has also been reported to influence cellulose digestion. Many different sample sizes have been utilized under various conditions and laboratory techniques. A preliminary factorial design with levels of .2, .4, .6, .8, and 1.0 grams alfalfa and .07, .12, .18, .24, and .30 grams solka floc were studied to determine the optimum level for cellulose digestion. Levels used in subsequent in vitro sums were .6 grams grass hays and .18 grams for solka floc (purified wood cellulose). Considering the forages studied, containing approximately 25-30 percent cellulose, these are roughly equivalent levels of cellulose. It should be noted that Quicke et al (72) found nearly the same percentage cellulose digestion for forage levels from .44 - 2.50 grams which represents cellulose levels of .08 - .48 grams,

C. Cellulose analysis - At the close of each fermentation

period, the tubes were immediately centrifuged at 2200 rpm for 8 - 10 minutes, the supernatent poured off, and the residual material refrigerated for subsequent cellulose analysis. Cellulose was determined by the method of Crampton and Maynard (26) with slight modifications. Samples were boiled for 40 minutes in the digestion reagent replacing the original 20 minutes, and hot benzene and ether washings were eliminated.

D. In vitro fermentation determinations - Solka floc tubes were added to each fermentation run to estimate between run variations. Several preliminary runs were made to investigate factors that may influence cellulose digestion under these conditions. Factors investigated were additions of glucose, urea, casein, B-vitamins (biotin and para-amino-benzoic acid), valeric acid, sample position in tank, particle size, substrate level, and use of two tubes in a line.

Rate of cellulose digestion for each hay and purified cellulose were studied by determination of percent cellulose digested after 3, 6, 12, 24, and 48 hour fermentations. Three replicates of this design were run and certain comparisons made with in vivo measurements.

RESULTS AND DISCUSSION

Coastal bermuda and blue panic are two forages that are high producing and have high carrying capacities when they are grown under proper fertilization and irrigation practices in southern Arizona and similar areas. However, animal performance has been relatively poor. Coastal bermuda has been evaluated as hay by Hammes et al (42) and Alexander et al (1,2), as "green chop" by Beardsley et al (11), and as pasture by Suman et al (80). Limited experimental work has been conducted with blue panicgrass, but field observations indicate that animal performance is similar. The above named hays along with alfalfa were evaluated here with the following results. Average daily dry matter intake per hundredweight (Table 2) was highest for alfalfa (3.36) and lowest for August cutting of blue panic (1.64). The bermuda dry matter consumption per hundredweight was .68, .86 pounds higher than panic although .54, .86 pounds less than alfalfa for the first and second cuttings, respectively. Percent dry matter digestibility (Table 3) was also highest on the alfalfa (61.5) as compared to 46.6 and 53.2 percent on the first cutting and 55.2 and 49.1 percent on the second cutting for bermuda and panic, respectively.

I. In Vivo Evaluation

A. Crude protein digestion and nitrogen balance data - The proximate chemical composition values shown in Table 4 suggested that all of the hays studied would be excellent sources of protein with the

TABLE 2

DAILY CONSUMPTION OF DRY MATTER, DIGESTIBLE DRY MATTER, DIGESTIBLE ENERGY AND DIGESTIBLE PROTEIN PER 100 POUND BODY WEIGHT

	Alfalfa	Blue Panic		Coastal Bermuda	
Cutting Dates	-	6-22	8-17	6-22	8-17
Dry matter, lb.	3.36	2.14	1.64	2.82	2.50
Digestible dry matter, lb.	2.07	1.14	0.80	1.31	1.37
Digestible energy, therms	4.32	2.27	1.84	2.74	2.44
Digestible protein, lb.	0.46	0.27	0.05	0.30	0.27

TABLE 3

APPARENT DIGESTION COEFFICIENTS, DIGESTIBLE ENERGY AND CALCULATED TDN VALUES

	Alfalfa	Blue Panic		Coastal Bermuda	
Cutting Dates	-	6-22	8-17	6-22	8-17
Apparent Digestion Coefficients:					
Dry matter, %	61.5	53.2	49.1	46.6	55.2
Gross energy, %	62.6	53.3	51.2	51.6	56.6
Crude protein, %	73.6	60.8	43.9	63.8	68.5
Digestible Energy and Protein Constant of Hays (Oven-Dry Basis):					
Digestible energy, therms per lb.	1.28	1.06	1.12	0.97	0.98
TDN[1], %	64.3	53.3	56.1	48.6	48.8
Digestible protein, %	13.5	12.8	2.9	10.5	10.7

[1]Calculated with 2000 kilocalories of digestible energy = 1 lb. TDN

TABLE 4

CHEMICAL COMPOSITION OF HAYS FED (OVEN-DRY BASIS)

	Alfalfa	Blue Panic		Coastal Bermuda	
Cutting Dates	-	6-22	8-17	6-22	8-17
Crude protein, %	18.4	21.0	6.5	16.5	15.6
Crude fiber, %	30.1	27.0	33.5	26.4	26.7
NFE, %	34.9	34.3	42.2	37.3	41.0
Ether extract, %	7.7	4.6	7.7	6.3	4.7
Ash, %	8.9	3.0	10.1	13.4	12.0
Gross energy, therms per lb.	2.807	1.995	2.020	2.090	1.982

exception of the August 17 cutting of blue panic. All hays provided more than an adequate amount of digestible protein (Table 2) for any productive level with the exception of the August cut blue panic. A significant ($P<0.01$) interaction for apparent digestion coefficients was found between hays (panic and bermuda) and cutting dates (June 22 and August 17). The interaction was primarily due to the drastic drop in apparent protein digestibility for the August cut blue panic while the digestible protein content of late cut bermuda increased slightly.

Nitrogen determinations were run on all feeds and excreta (Table 5). Nitrogen excreted in the urine was fairly constant for all hays fed, excluding the August cutting of panic in which there was a marked reduction in urinary nitrogen excretion. However, this was compensated for by the elimination of more apparently undigested protein (nitrogen) in the feces. This same interaction, as mentioned with apparent digestion coefficients for protein, was significant ($P<0.01$) for the analysis of variance of nitrogen retention.

TABLE 5

NITROGEN BALANCE STUDY (4 LAMBS PER TREATMENT)

	Alfalfa	Blue Panic		Coastal Bermuda	
Cutting Dates	-	6-22	8-17	6-22	8-17
N fed, gm.	144.3	159.4	46.6	109.9	119.1
Fecal N excreted, gm.	38.4	62.5	26.5	39.8	37.4
Percent of total, %	26.6	39.2	56.9	36.2	31.4
Urinary N excreted, gm.	74.5	70.6	13.3	60.0	58.8
Percent of total, %	51.6	44.3	28.5	54.6	49.4
N retained, gm.	31.4	26.3	6.8	10.2	22.8
Percent of total, %	21.8	16.5	14.7	9.3	19.2

B. Gross energy digestion and consumption - No significant differences in gross energy digestibilities were found between blue panic and bermuda at either cutting date (Table 3). However, 24 and 34 percent more coastal bermuda dry matter was consumed per 100 pounds of body weight than blue panic for the June and August cuttings, respectively. A more rapid rate of passage of coastal bermuda dry matter through the intestinal tract was suggested by the greater dry matter intake (Table 2) and lower digestible energy content per pound of bermuda-grass hay at both cutting dates.

The increased feed intake for the coastal bermuda fed lambs resulted in approximately 500 more kilocalories of digestible energy intake per 100 pounds of body weight as compared to the blue panic fed lambs on both cutting dates.

The high quality alfalfa hay was found to be much superior to either of the grass hays in every comparison of nutritive value made.

II. *In Vitro* Evaluation

After a preliminary screening of substrate levels, .6 and .8 grams of alfalfa and .12 and .18 grams solka floc were run in a three-way factorial design with replications and fermentation lengths as other factors. Analyses of data showed no significant differences in percentage cellulose digestion with either level of alfalfa or solka floc. Therefore, .6 and .18 levels were used for subsequent *in vitro* fermentations for hays and solka floc, respectively. These two levels were chosen since the hays contained approximately 25 - 30 percent cellulose, thus resulting in equivalent cellulose concentrations. Significant differences were found between replications of the design (Table 10) where replication represented separate runs. Therefore, solka floc was incorporated in all fermentation runs to check the repeatability of results between runs as well as to allow comparisons with other laboratories. Replication by treatment interaction for alfalfa, replication by treatment, and replication by level interactions were significant with solka floc. The other two-way and higher order interactions were non-significant. Coefficients of variations were approximately 2 percent. The grinding of forage through 40 mesh screen with a laboratory wiley mill improved the rate of cellulose digestion. Sample position in the tank was found to have no influence on cellulose degradation.

The means of the hays and solka floc *in vitro* fermentations are shown in Table 6, with individual values in Table 9 and fermentation curves plotted in Figure 1. It can be seen from Table 9 values that the 3 and 6 hour determinations produced greater variability

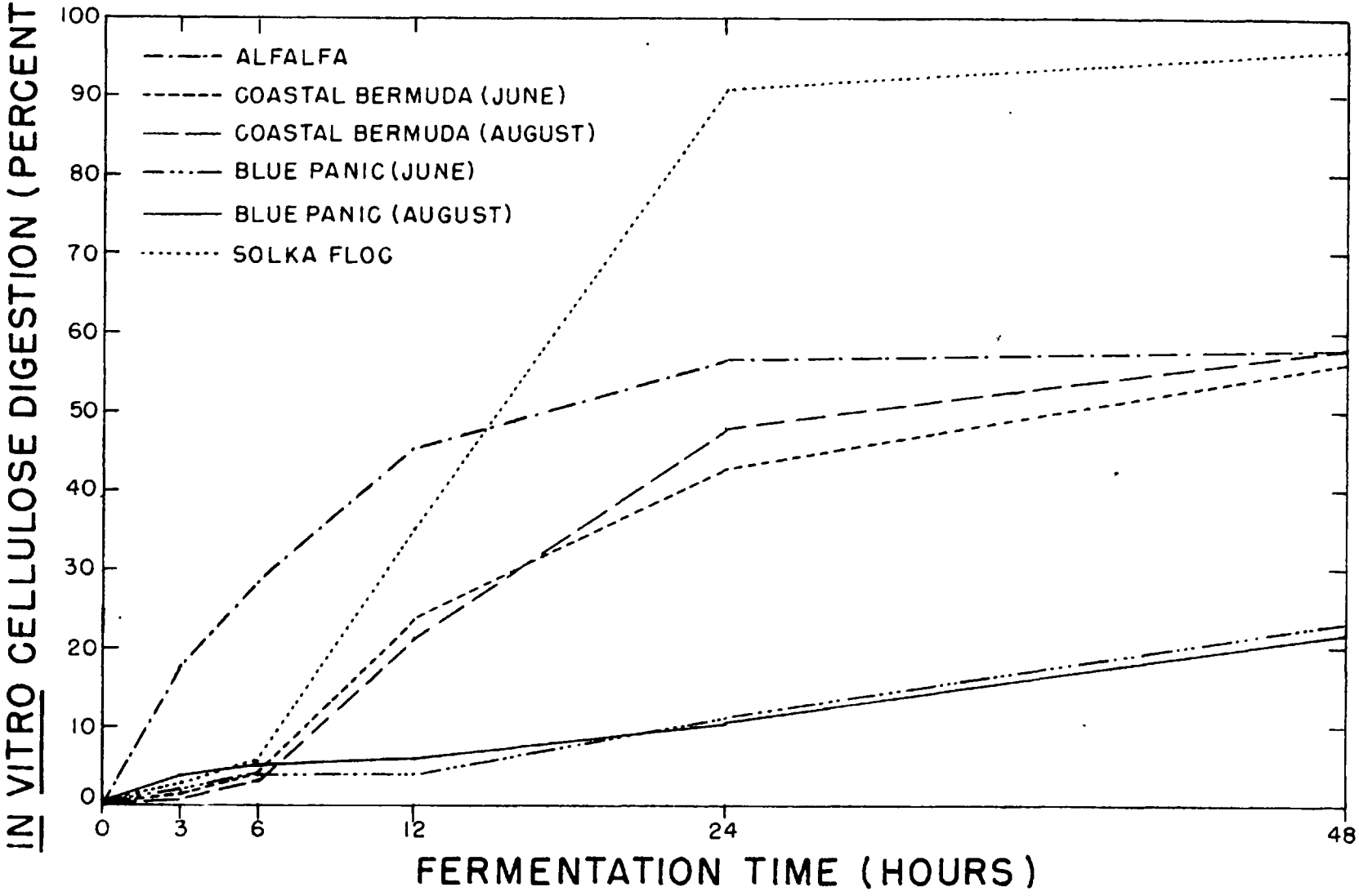

Figure 1.- Rate of in vitro cellulose digestion, 1960 Forages and Solka Floc.

TABLE 6

AVERAGE IN VITRO CELLULOSE DIGESTION VALUES[1]

Hays	Fermentation Time (Hours) 3	6	12	24	48
	(%)	(%)	(%)	(%)	(%)
1. Alfalfa	17.87	28.21	45.05	56.45	58.00[7]
2. Blue Panic (June)	2.07[2]	3.89[3]	3.82[4]	11.00[6]	23.01[8]
3. Blue Panic (August)	4.02[2]	5.09[3]	5.81[4]	10.64[6]	21.91[8]
4. Coastal Bermuda (June)	1.42[2]	4.00[3]	23.77[5]	42.49	56.24[7]
5. Coastal Bermuda (August)	.85[2]	3.08[3]	20.91[5]	48.07	58.14[7]
6. Solka Floc	2.83[2]	6.01[3]	34.34	90.54	96.18

[1]Means with the same superscript are not significantly different ($P < .05$).

between replications, yet relative differences between hays and solka floc within a replication (run) remained rather constant. This evidence indicates the value of in vitro technique, not so much in establishing hard and fast values for each forage, but rather as a method for comparisons of hays and establishing the relative value of each.

Figure 1 represents an overall view of each of the 5 hays and solka floc (control) that was studied. Wide differences in hays can immediately be recognized solely from the rate of in vitro cellulose

fermentation data presented. The general characteristics represented by these curves are similar to those obtained by Herschberger et al (44) and Donefer et al (31).

Analyses of hays and solka floc by fermentation periods (Table 6) showed hays to be significantly different from each other ($P<0.01$) in all cases. Replications were significantly different at the .01 level for 6 and 24-hour fermentation length, however, only at the .05 level for the remaining 3 and 48-hour fermentation periods. The Duncan Multiple Range Test (Table 6) detected no differences between bermuda, panic, and solka floc at 3 or 6 hour intervals; however, percent cellulose digestion of alfalfa was significantly different from all others at both time periods. At the end of the 12-hour in vitro fermentation, four distinct classes were separable: blue panic, coastal bermuda, solka floc, and alfalfa. There were no significant differences between cutting dates within either species. At the end of the 24-hour analysis, detectable differences were found between June and August coastal bermuda in addition to those significant differences observed at the end of the 12-hour period. Between the 24 and 48-hour interval, the rate of cellulose digestion was considerably slower than the previous interval; however, the rate of alfalfa cellulose digestion was very low, almost allowing coastal bermuda to reach it by the end of the 48-hour and final period studied.

It can also be noted from observing the graph (Figure 1) that grasses and solka floc lagged behind the alfalfa in the initial periods of fermentation and in the case of blue panic grasses never approached the others throughout the trial. This agrees quite well

with observations by Baumgardt et al (10) and Donefer et al (31) who reported initial rates of cellulose digestion greater and the maximum digestion obtained sooner for alfalfa than with grasses. It may be that the fermentation of unlignified, purified cellulose (90 percent digested in 24 hours) is closely related to the grasses in the early stages of fermentation, but unlike them, there is no decrease in rate of digestion until it is almost completely digested. Donefer et al (31) suggested that the decrease in rate of digestion after 12 hours is related to lignification of the forage. Kamstra et al (52) and later Sullivan (78) found, by separating cellulose from lignin, the inhibiting effect of lignin upon cellulose digestion disappeared. Donefer et al (31) has also shown that the major amount of digestion is accomplished in the first 24 hours with only a small increase in the last half of the incubation period (Figure 1).

By comparing the hays in the light of their chemical composition, we can only hypothesize as to other factors responsible for the differences observed in the rate of cellulose digestion. Composition and concentration of ash and added fat seem to be factors worthy of further investigation.

The in vivo and in vitro measurements along with calculated In Vitro and Nutritive Value Indices are shown in Table 7. Each in vivo value is the average of four animals, whereas in vitro measurements are based on three separate fermentation runs. There was less variability among in vitro fermentation replications (Table 9) than among animals. Refer to Appendix for Table 9.

All possible correlation coefficients were calculated between

TABLE 7

IN VITRO AND IN VIVO MEASUREMENTS USED IN CALCULATIONS OF IN VITRO INDEX AND EFFECTIVE NUTRITIVE VALUE INDEX

Forage	In Vitro Measurements			In Vivo Measurements		
	Cellulose Digestion					
	12 hr.	24 hr.	IVI[1]	RI[2]	DE[3]	NVI[4]
	(%)	(%)			(%)	
Alfalfa	45.0	56.5	25.4	109.0	62.6	68.2
Blue Panic (June)	3.8	11.0	4.2	69.4	53.3	37.0
Blue Panic (August)	5.8	10.6	6.1	53.2	51.2	27.2
Coastal Bermuda (June)	23.8	42.5	10.1	91.5	51.6	47.2
Coastal Bermuda (August)	20.9	48.1	10.1	81.1	56.6	45.9

[1] 12x24 hour *in vitro* cellulose digestion values = *in vitro* index (IVI).

[2] Relative intake values as proposed by Crampton *et al* (25).

[3] Digestible energy (%).

[4] Nutritive value index (NVI) = Relative intake x Digestible energy(%).

TABLE 8

SIMPLE AND MULTIPLE CORRELATIONS OF *IN VITRO* AND *IN VIVO* DATA

Variables Correlated	Coefficients	
Simple Correlations	r	r^2
A. Relative intake		
3 hr. *in vitro* cellulose digestion	.63	.39
6 hr. " " " "	.71	.50
12 hr. " " " "	.94	.88
24 hr. " " " "	.89	.80
48 hr. " " " "	.85	.72
B. Digestible energy		
3 hr. *in vitro* cellulose digestion	.84	.71
6 hr. " " " "	.89	.80
12 hr. " " " "	.86	.74
24 hr. " " " "	.75	.56
48 hr. " " " "	.58	.34
C. Nutritive Value Index		
12 hr. accumulative area under curve	.78	.60
24 hr. " " " "	.95	.89
48 hr. " " " "	.53	.28
D. Nutritive Value Index		
12 hr. *in vitro* cellulose digestion	.95	.90
12 x 24 hr. *in vitro* cellulose digestion (In Vitro Index)	.92	.85
24 hr. *in vitro* cellulose digestion	.87	.76
Multiple Correlations		
A. Nutritive Value Index		
12 hr. *in vitro* x 24 hr. *in vitro* cellulose digestion	.95	.90

[1]Accumulative area under curve obtained by adding total number of squares under each *in vitro* fermentation curve with use of ordinary graph paper.

in vitro and *in vivo* data to determine possible relationships existing between the rate of *in vitro* cellulose fermentation and *in vivo* measurements of nutritive value for the same forages (Table 8). Since the Nutritive Value Index (NVI) is calculated from relative intake and digestible energy values *in vivo*, these correlations were also studied with *in vitro* data. Relative intake measurements were found to be most highly correlated with *in vitro* values. More specifically the 12 hour *in vitro* cellulose digestion was correlated to the extent of .94 with relative intake (Voluntary Consumption), as shown in Table 8.

This confirms earlier reports of the existence of a relationship between the rate of forage cellulose digestion during the first 12 hours of *in vitro* fermentation period and the voluntary *in vivo* consumption of the forage as shown by Donefer *et al* (31) and Crampton *et al* (25). Other *in vitro* values were also correlated with NVI's. Donefer *et al* (31) found 12 hour *in vitro* digestion to be more effective in predicting the NVI than *in vitro* index (12 x 24 hr. values). This agrees with data reported here also. However, an additional calculation of accumulative area under the curves (Figure 1) are made showing 24-hour accumulative area values to be correlated equally as well as 12-hour percent cellulose digestion with NVI (Table 8). This later observation suggests another use for 24 hour data other than for digestible energy calculations as has been shown and confirmed by previous *in vitro* studies.

Since 12-hour *in vitro* cellulose digestion values (X) were one of the highest correlations observed with NVI, it has been proposed that it be used to estimate NVI (Y) of forage (Figure 2). On

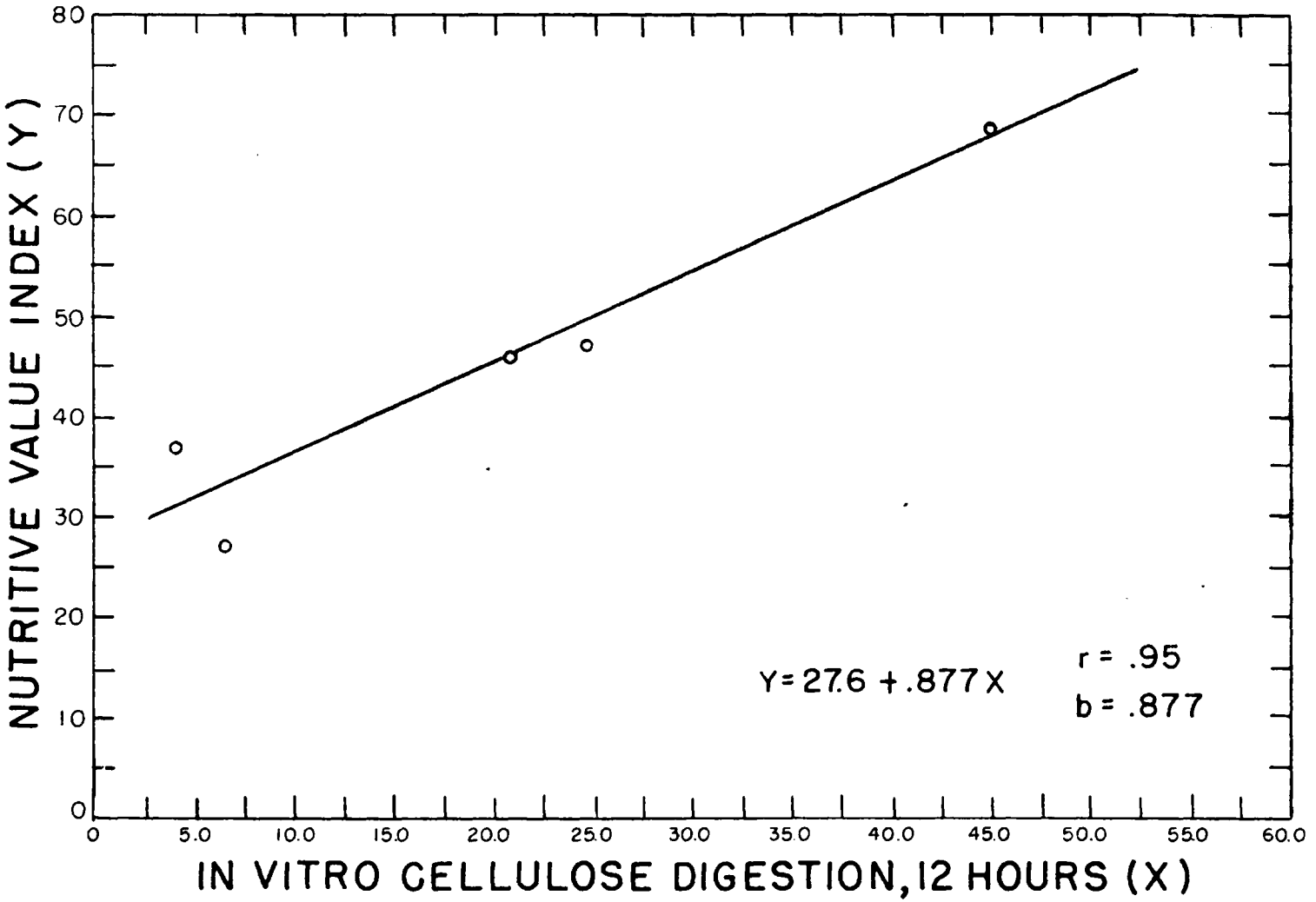

Figure 2.- Regression of nutritive value index on 12 hour in vitro cellulose digestion.

this basis the regression equation obtained here is $Y = 27.6 + .877X$. Although these two variables are highly correlated, the regression equation expressed here is not based on enough different forages to be of concrete value. Nevertheless, it serves as an example of what can be done for NVI prediction of a forage by use of only the 12-hour *in vitro* values which are relatively easy to obtain.

SUMMARY

Coastal bermuda and blue panic hays harvested on two cutting dates, June 22 and August 17, were compared with alfalfa (second cutting) as a control. Wethers were used as experimental animals for palatability (6 per treatment), digestibility and nitrogen balance studies (4 per treatment). Rendering plant tallow was added to hays before grinding to control dust. Chemical analysis showed all hays to be excellent protein sources with the exception of the August 17 cutting of blue panicgrass. There was a significant interaction between hays and cutting dates for apparent protein digestion and nitrogen retention. Ad Libitum digestible dry matter intake (lbs. per 100 lbs. body weight) was as follows: Alfalfa, 2.07; June 22 - bermuda, 1.31; June 22 - blue panic, 1.14; August 17 - bermuda, 1.23; and August 17 - blue panic, 0.91. No significant differences in grass energy digestibilities were found between the grasses at either cutting date. However, the lambs fed on the bermuda grass consumed .7 and .9 pounds more dry matter per 100 pounds of body weight than those on panicgrass for June and August cutting, respectively. This greater dry matter intake indicates a more rapid rate of passage of coastal bermuda grass. Alfalfa exceeded the grass hays in all comparisons made.

The second phase consisting of cellulose determinations run at the end of 3, 6, 12, 24, and 48 hours of in vitro fermentation with a

mixed suspension of rumen microorganisms verified to great extent the results previously observed *in vivo*. Analyses of hays and solka floc (purified wood cellulose) by fermentation period showed treatments to be significantly different in all cases. No detectable differences were found between grasses and solka floc until the end of the 12-hour incubation period. Here the unlignified purified cellulose was well ahead of grasses but not digested to the extent of alfalfa cellulose. No cutting differences could be observed, but there were significant differences between alfalfa, solka floc, bermuda, and panicgrasses. Bermuda grass, as is typical of grasses, lagged behind the legume in initial periods of fermentation; however, it was significantly ahead of panicgrasses by the end of the 12-hour period. By the end of the 24-hour period the only other significant differences observed were between the bermudas which vanished at the end of the 48-hour period. However, by this period the cellulose digestion of the bermuda nearly equalled that for alfalfa with the panicgrasses still lagging well behind. There was less variability among *in vitro* replications than the *in vivo* digestion trials, indicating repeatability of *in vitro* fermentation procedure. The reported relationship between the rate of forage cellulose digestion during the first 12 hours of *in vitro* fermentation period and the voluntary *in vivo* consumption of the forage was confirmed ($r = .94$). Equally high correlations were obtained between the 12-hour *in vitro* cellulose digestion, or 24-hour accumulative digestion values (area under the curve), and Nutritive Value Indices.

APPENDIX

TABLE 9

INDIVIDUAL IN VITRO CELLULOSE DIGESTION VALUES

Hays	Fermentation Time (Hours)				
	3	6	12	24	48
	(%)	(%)	(%)	(%)	(%)
Alfalfa	17.52	32.25	44.86	54.87	59.64
	8.59	22.89	41.83	57.47	59.69
	27.50	29.49	48.43	56.99	54.68
Blue Panic (June)	3.20	6.65	3.07	10.12	21.86
	0.00	1.18	3.54	10.24	25.08
	3.01	3.84	4.86	12.65	22.11
Blue Panic (August)	4.11	6.91	3.30	9.29	27.87
	0.00	0.00	2.49	10.30	18.13
	7.96	8.35	11.65	12.34	19.72
Coastal Bermuda (June)	2.13	5.44	19.96	40.95	56.09
	0.00	.38	25.80	42.34	60.27
	2.13	6.18	25.55	44.17	52.35
Coastal Bermuda (August)	.38	2.70	19.83	46.90	57.36
	0.00	2.32	21.47	49.06	60.87
	2.18	4.23	21.43	48.26	56.18
Solka Floc	3.98	6.39	23.81	90.45	96.60
	1.77	4.78	38.42	89.76	95.90
	2.74	6.86	40.79	91.42	96.05

TABLE 10

ANALYSES OF VARIANCE AND COEFFICIENTS OF VARIATION FOR ALFALFA AND SOLKA FLOC IN VITRO CELLULOSE DETERMINATIONS

Source of Variation	Degrees of Freedom	Mean Squares	
		Alfalfa	Solka Floc
Replications (Runs)	1	217.56[1]	123.77[1]
Treatments (12 hr. vs 24 hr.)	1	462.25[1]	12,337.66[1]
Level (.12 gms. vs .18 gms.)		-	.03
(.6 gms. vs .8 gms.)		1.32	-
Replication by Treatment	1	30.81[1]	25.75[1]
Replication by Level	1	.82	8.71[1]
Treatment by Level	1	2.73	.43
Treatment by Replication by Level	1	.34	3.00
Error	8	.945	.723
Coefficient of Variation		2.07%	1.90%

[1] ($P < .01$)

TABLE 11

ANALYSES OF VARIANCE AND COEFFICIENTS OF VARIATION FOR IN VITRO PERCENT CELLULOSE DIGESTION DETERMINATIONS

Source of Variation	d.f.	Mean Squares for Fermentation Periods (Hrs.) 3	6	12	24	48
Replications	2	52.08[2]	43.93[1]	59.79	7.31[1]	19.21[2]
Treatments	5	125.85[1]	286.25[1]	767.61[1]	2,722.56[1]	2,280.67[1]
Error	10	12.12	3.76	14.80	.71	4.67
Coeff. of Variation		71.90	44.87	17.28	2.01	4.13

[1] ($P \leq .01$)

[2] ($P \leq .05$)

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