EFFECT OF VARIOUS COMBINATIONS AND PROPORTIONS OF FEEDSTUFFS WITH AND WITHOUT AUREOMYCIN ON THE IN VITRO DIGESTION OF CELLULOSE BY RUMEN MICROORGANISMS

by

Frank John Hanold

B. S., Kansas State College
of Agriculture and Applied Science, 1952

- - - - - - - - - -

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

TABLE OF CONTENTS

INTRODUCTION

An appreciation of the importance of rumen microorganisms has led to the development of several laboratory techniques for studying their nutrition under conditions simulating those within the bovine rumen. By the use of these techniques, it has been possible to investigate the importance of individual nutrients such as nitrogen, minerals, vitamins, fatty acids, unidentified factors, and individual feedstuffs or combinations thereof in the nutrition of rumen microorganisms.

While the effect of various nutrients and individual feedstuffs on the *in vitro* digestion of cellulose by rumen microorganisms has been studied, there is a paucity of information on the influence of combinations of feedstuffs as they occur in practical farm rations. Also, the effect of single grains, as compared to mixtures of grains on the digestion of cellulose has never been studied. Accordingly, work was initiated to investigate the effects of various combinations of some commonly used feedstuffs on the *in vitro* digestion of cellulose.

Since pure crystalline aureomycin[1] was shown to depress cellulose digestion *in vitro*, and since certain nutrients were shown to alleviate this depression better than others, it was hoped that the inclusion of this antibiotic would magnify the differences in the effectiveness of rations by imposing a stress on the microorganisms. Also, it was desired to ascertain whether certain feed combinations would more adequately overcome the stress than other combinations.

---

[1] The Trademark of Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., for the antibiotic chlortetracycline.

Tremendous quantities of poor quality roughage are produced annually and fed to livestock. A slight improvement in the efficiency of utilization of such feed would increase its feeding potential and be of considerable economic importance to the livestock industry. For this reason, and in order to demonstrate wider differences in digestibility resulting from additions of certain feedstuffs, prairie hay was used as the test roughage.

It is hoped that the more promising results that may evolve through this preliminary screening by the artificial rumen technique may be duplicated in live animal experiments.

REVIEW OF LITERATURE

The Effect of Various Nutrients on the *In Vitro* Digestion of Cellulose

In Vitro Techniques. The cost and time involved in conducting digestion studies within the live animal brought about the development of a method by Marston (1948), which could be used to study the *in vitro* digestion of feedstuffs by rumen microorganisms. It was intended that this method might serve as a screening device, thereby avoiding unnecessary preliminary research. However, he felt that conclusive results would have to be obtained by repeating with the live animal the most promising *in vitro* work. In the procedure of Marston (1948), rumen liquor was used. It was obtained by pressing semisolid rumen material through a sieve into a glass container in which the air had previously been replaced by carbon dioxide. Precautions were taken to minimize heat losses and to insure, as nearly as possible, anaerobic conditions throughout the procedure. A predetermined quantity of substrate and rumen liquor was incubated in a 3.5 liter glass container fitted with a metal lid which could facilitate a continous flow of carbon dioxide and the periodic regulation of pH. A satisfactorily constant pH of 6.8 was maintained by conducting the fermentation under the influence of certain salts of high buffering capacity. These salts were added to the glass container at the start of the fermentation period, and as the pH dropped sufficiently to warrant further additions. The flasks were kept in a water bath maintained at 40° C. At the end of 24 hours the contents were made up to 50 ml. volumes which were used for the determination of residual cellulose.

A similar method was described by Louw, et al. (1949) in which the inoculum and substrate were placed in a "Visking" cellulose sausage casing of 4 1/3 inch diameter. This was then suspended in a glass jar containing a buffer solution. The fermentation products which normally interfere with the digestion of cellulose could then dialyse through the semi-permeable cellulose casing and provide for a more natural environment within the membrane. All other conditions were similar to those mentioned by Marston (1948). The method of Louw, et al. resulted in greater digestion of cellulose. They postulated that the poorer digestion observed by Marston was due to an inhibitory influence of fission products accumulating in the glass bottle not containing the semi-permeable cellulose casing.

Arias, et al. (1951) introduced several modifications of the procedure of Marston (1948). In their method, a series of 10 consecutive 24-hour fermentations were used. Six flasks each in two water baths were connected to enable simultaneous studies under the influence of portions of the same inoculum. At the end of each 24-hour fermentation period the contents of the flasks were individually divided into two equal portions, one for analysis purposes, and the other used as inoculum for the subsequent 24-hour period. They believed that this would progressively dilute any nutrients that might appear in the original inoculum. Therefore they only gave consideration to the last five 24-hour fermentation periods.

In order to facilitate more routine in vitro determinations of digestion by rumen microorganisms, Huhtanen, et al. (1954) developed a "miniature artificial rumen". A small cellophane sac was suspended in a four ounce screw-cap jar containing a solution similar in mineral composition to sheep saliva. The mineral solution, due to its high buffering capacity, allowed the inhibitory end-products of microbial digestion to dialyze through the sac and thereby provide for a more natural environment within the membrane for a longer period of time. This technique exemplifies the three major advantages which Burroughs attributed to the artificial rumen in general: (1) speed, (2) precision, and (3) minimum expense.

Marston (1948) stated that "The environment in the rumen is the main factor which regulates the community of microorganisms responsible for carbohydrate degradation." He further stated that "The low partial pressure of oxygen and the high CO_2 tension, the supply of phosphate and inorganic nitrogen from the saliva, the buffering capacity of the saliva, the temperature of approximately 40° C., and the residual contents of the rumen, which retain a massive inoculum of a community of microorganisms are apparently the main factors which determine the course of fermentation in the normal rumen." All of these factors were either supplied by the inoculum or were maintained throughout the fermentation in each of the methods discussed above for in vitro digestion of cellulose by rumen microorganisms. Qualitative differences of the microbiota obtained from the live animal appear to be limited, as reported by Gall, et al. (1949), since they found that differences exist in the predominating types of rumen microorganisms present under different feeding regimes but these differences were more quantitative than qualitative. That is, the same general types of organisms were present in each animal even though the predominating type was different.

Effect of Feedstuffs on the In Vitro Digestion of Cellulose. The effect of good and poor quality roughages was studied by Burroughs, et al. (1950b), in several series of *in vitro* fermentations. Alfalfa hay, red clover hay and rye hay served as good quality roughages. Corn stover, wheat straw, corncobs and timothy-blue grass hay were used as poor quality roughages. It was intended to demonstrate that efficiency of roughage digestion is dependent upon the presence or absence of essential nutrients in the roughage that are required for maximum functioning of rumen microorganisms. A complex mineral mixture was included in one fermentation series. The complex mineral mixture plus water extract of cow manure was included in a second series, and available nitrogen plus the complex mineral mixture and water extract of cow manure in a third series of fermentations. With poor quality roughage, Burroughs, et al. (1950b) found that available nitrogen alone, or in combination with the complex mineral mixture, would not sustain a satisfactory digestion. However, they found a very efficient digestion during all 10 periods when an autoclaved extract of manure was added to the other nutrients. The cellulose in the good quality roughage was digested efficiently without supplementation. As a result of this work, Burroughs, et al. concluded that the types of nutrients required by rumen microorganisms fall into three general classifications: (1) energy which is the motivating force, (2) protein or its elements such as nitrogen, and (3) inorganic constituents which play an important role in enzymes, enzyme systems and vitamin synthesis.

Burroughs, et al. (1950a) gave consideration to two main categories of factors involved in microbiological digestion:

(1) Conditions which are fixed or regulated by the physiology and anatomy of the host animal such as temperature, moisture, salts contained in saliva, anaerobiosis, absorption of organic acids through the rumen wall, motion or stirring and possibly exclusion of light.

(2) Influential conditions subject to variation related directly to the environment of rumen microorganisms or indirectly to the environment of the host animal. His examples of these conditions were the types of microorganisms present in the rumen or inoculum of the fermentation flask, the nutrients available to the bacteria, properties of the medium such as pH, total acidity, total salt concentration, and possible symbiotic relations between the different types of organisms present.

In some further work by Burroughs, et al. (1950c), the same basic constituents of available nitrogen, complex mineral mixture, and cow manure extract were compared when grains and protein-rich feed were added. With ammonium sulphate, they found an increase in the ability of rumen microorganisms to digest cellulose with distillers solubles, linseed meal, soybean oil meal and to a slight extent with meat scraps. There was no increase with dried skimmilk, molasses, corn, wheat bran, cottonseed meal, fish meal, liver meal and oats. When ammonium sulphate plus complex minerals were added, all but meat scraps, fish meal, liver meal and oats increased cellulose digestion. Stimulatory influences were assigned positive values representing the difference between the control flasks and the supplemented flasks in regard to cellulose digestion. The grains which showed the greatest influence were dried distillers solubles, linseed meal, and soybean oil meal. Those of intermediate influence were dried skimmilk, molasses, corn, wheat bran, and cottonseed meal. Little or no influence resulted from the addition of meat scraps, liver meal, and ground oats. From this work, Burroughs, et al. (1950c) concluded that certain cereal grains, and protein-rich feeds, influence micro-biological digestion of cellulose but the amounts needed cannot be accurately determined from the present data. They concluded that the evidence indicated the mode of action is through nutrients the feeds supply to cellulose-splitting rumen microorganisms. This conclusion is in agreement with a statement made by Hungate (1950) that " - in approaching the problem of nutritional requirements of rumen microorganisms it should be kept in mind that the first step in ruminant nutrition is microbial nutrition."

Burroughs, et al. (1951a) also compared the influence of a series of both purified proteins and a series of protein-rich feeds, with and without urea on the digestion of cellulose in filter paper. The purified proteins consisted of casein, blood fibrin, lactalbumin, zein, egg albumin and wheat glutin. The protein-rich feeds included fish meal, soybean oil meal, wheat gluten, meat scraps, linseed meal and cottonseed meal. This study approached the utilization of cellulose from the standpoint of the availability of nitrogenous compounds as compared with protein-rich feeds in supplying one of the three general nutrient requirements, protein or its elements, mentioned previously by Burroughs, et al. (1950b). It was concluded from this work that the nitrogenous requirements of rumen microorganisms are relatively simple, involving ammonia and not the more complex forms of nitrogen such as amino acids. Burroughs, et al. (1951a) concluded that proteins may aid micro-biological digestion of cellulose by contributing a mineral element such as phosphorous or sulphur and by supplying minerals that aid cellulose digestion they thus unlock additional energy for rumen bacterial development. Finally, the energy content of protein itself may aid cellulose digestion.

A report by Arias, et al. (1951) demonstrated that starch significantly depressed cellulose digestion. This was confirmed by Hunt, et al. (1954) and Radisson (1955), and bears out the fact, as stated by Arias, et al. (1951) that " - the more readily available source of energy will be primarily attacked by the rumen microorganisms and the cellulose secondarily." Hunt, et al. (1954) further showed that fermentation time had a highly significant effect on the digestion of cellulose. Furthermore, he showed that more cellulose was digested when the inoculum was obtained from a steer fed alfalfa hay than from one receiving poorer quality hay. Statistically, the differences found were highly significant. It was postulated that the alfalfa hay ration supplied nutrients for the microorganisms which stimulated their activity or supported a greater population while the poor hay did not.

Effect of Urea on the In Vitro Digestion of Cellulose. Radisson (1955) found that small amounts of urea (2.5 to 5.0 mg.) were stimulatory to cellulose digestion but that larger amounts (10 to 50 mg.) of urea depressed cellulose digestion in the artificial rumen. Belasco (1954) reported that the amides of monocarboxylic acid produced a smaller response than that noted with urea. He also found that the cellulose digestion which occurred with certain amidines and ammonium salts was comparable to that found with urea.

The influence of urea at a constant level with different amounts of good and poor quality proteins in regard to cellulose digestion was studied by Burroughs, et al. (1951a). Casein was used as the source of good quality protein and gelatin served as a source of poor quality. Their artificial rumen contained a low, medium and high level of each of these proteins. The flask containing the low amount of casein showed favorable cellulose digestion as compared with the flask having the low amount of gelatin. At higher levels of proteins this relationship was reversed. This work also demonstrated that cellulose digestion was higher (averaging 66%) when urea was used in the experimental flasks than when urea was not used (averaging 48%).

Effect of Minerals on the In Vitro Digestion of Cellulose. In studying mineral influences upon cellulose digestion by rumen microorganisms, Burroughs, et al. (1951b) found that water extracts from dehydrated clover meal, rumen ingesta, and manure, each stimulated cellulose digestion. He then fractionated blackstrap molasses into organic and inorganic substances and found the ash stimulated cellulose digestion to the same extent as did molasses. Adding sugar to molasses ash resulted in about the same digestion as with molasses or with molasses ash and distinctly better digestion than in the control flask, or in the flask containing only the sugar addition. The amount of molasses ash required for efficient cellulose digestion was more dependent upon the amount of available energy present than upon the amount of cellulose present. Similar results were obtained from the addition of clover hay ash or mature timothy hay ash when added in amounts similar to the amount of molasses ash added.

It was felt by Burroughs, et al. (1950a) that the nutrients available in and other characteristics of the feed and water contained in the fermentation medium probably exert the major influences upon cellulose breakdown by rumen microorganisms. This reasoning brought about a study by Burroughs, et al. (1950a) through which they found that substances in autoclaved rumen liquid and autoclaved extract from cow manure stimulated the digestion of cellulose by rumen microorganisms. They also found that minerals from different sources appeared to be beneficial to rumen microorganisms in digesting fiber. This tends to confirm the beneficial influence of alfalfa ash upon roughage digestion, previously found by Burroughs, et al. (1950b).

Burroughs (unpublished data) indicated that a water extract from dehydrated alfalfa enhanced the dry matter digestibility of a poor quality roughage. However, grass juice was found by Radisson (1955) to depress cellulose digestion when used at high levels, but low levels were stimulatory.

Burroughs, et al. (1951b) also suggested that iron and phosphorous aided cellulose digestion. A stimulation resulted from the addition of iron in the absence of molasses ash. Several assumptions resulted from this work: (1) a roughage could be utilized most efficiently when each of the nutrient requirements, energy, protein or its elements, and minerals were adequately supplied, (2) the mineral requirements of rumen microorganisms for maximum functioning appear to be many in number, (3) the availability of ashes as well as the quantity of ash from different quality hays may vary with the maturity of the plant, and (4) certain mineral additions such as iron and phosphorous to molasses feeds appear justified but are worthy of additional study.

Sulphur as found in methionine and sodium sulphate was found by Hunt, et al. (1954) to stimulate the digestion of cellulose to a greater extent than cystine or elemental sulphur. It appeared from this work that elemental sulphur cannot be used by microorganisms involved in the breakdown of cellulose. Evidence of a symbiotic relationship was presented in this report. Also, it was postulated that pantothenic acid, which appears to be synthesized by certain non-cellulose splitting microorganisms, may be utilized by the microorganisms which digest cellulose.

Effect of Unidentified Factors and Vitamins on the In Vitro Digestion of Cellulose. Bentley, et al. (1954a) reported that water extracts of certain plant materials increased cellulose digestion by means of a factor which was deficient in the basal medium, and which could be supplied by alfalfa leaf meal, molasses, yeast and rumen juice. He also found that a combination of adenine, uracil, xanthine, nine B-vitamins and molasses ash or alfalfa ash improved cellulose digestion. Combinations of either of these ashes or purines and uracil, with three B-vitamins, gave better results than the B-vitamins alone. A retardation of cellulose digestion was attributed to an inability of the microorganisms to synthesize biotin, paraaminobenzoic acid, and vitamin B_{12} for optimum growth in the absence of rumen juice. Synthesis of these stimulatory compounds from simple nutrients, such as ammonia, salts, and a carbon source, was thought to be inadequate. Bentley, et al. (1954a) further demonstrated that the additions of biotin, paraaminobenzoic acid, vitamin B_{12} along with purine and pyrimidine bases, greatly enhanced cellulose digestion.

Effect of Fatty Acids on the In Vitro Digestion of Cellulose. Bentley, et al. (1954b) also reported that "A volatile fraction from acidified rumen juice and various straight and branched chain fatty acids have cellulolytic factor activity for rumen microorganisms in vitro." Caproic and n-valeric acids were the most active although iso-valeric and iso-butyric acids were found to have some stimulatory effect. Preliminary evidence also indicated, α - amino homologs of the volatile fatty acids are also active in stimulation of cellulose digestion. Bentley, et al. (1954b) postulated that this may be the factor(s) which are responsible for the factor activity found in certain feeds, yeast, and yeast by-products.

A factor, or factors, that stimulates the growth and cellulolytic activity of rumen microorganisms has been obtained in crude form by Garner, et al. (1954). The factor was found in active extracts from bovine ingesta, ovine ingesta, and two fermented feeds that were free of microorganisms.

Effect of Antibiotics on the In Vitro Digestion of Cellulose. Wasserman, et al. (1952) and Radisson (1955) studied the effect of antibiotics on the *in vitro* digestion of cellulose by rumen microorganisms. Penicillin stimulated cellulose digestion at low concentrations, as reported by Wasserman, et al. (1952), but depressed cellulose digestion at high concentrations as shown by Radisson (1955). Both of these workers found neomycin to be stimulatory, whereas there was a divergence of opinion as to the effect of streptomycin. Radisson, (1955) found that tyrothrycin, gramicidin, penitracin and dihydrostreptomycin sulfate had no significant effect on cellulolytic activity of rumen bacteria. He also stated that " - aureomycin increased the requirement of rumen bacteria for a factor(s) not normally needed, or needed in much smaller amounts for cellulolytic activity in the absence of aureomycin. Alfalfa hay, grain, urea, and soybean oil meal each were found to be good sources of this factor(s) since they either reduced or reversed the inhibitory effect of aureomycin on digestion of cellulose *in vitro*." Radisson (1955) found a greater stimulation of cellulose digestion from either alfalfa hay, grain, urea or soybean oil meal in the presence of aureomycin than in the absence of aureomycin. It was further observed by this worker that whereas the addition of aureomycin to the artificial rumen severely inhibited cellulose digestion by microorganisms obtained from the rumen of control animals, it had only a slight effect on the cellulolytic activity of rumen microflora of aureomycin-fed calves. He concluded that the bacteria became adapted to aureomycin.

Effect of Steroid Compounds on the In Vitro Digestion of Cellulose. The effect of some steroid compounds on cellulose digestion was studied by Brooks, et al. (1954). Stilbestrol was tested at levels of 2, 8, 10, 16, and 20 micrograms per artificial rumen. Stilbestrol added at the rate of 2 to 8 micrograms increased cellulose digestion slightly. The addition of stilbestrol at the rate of 16 to 20 micrograms resulted in a significant ($P > 0.05$) increase in cellulose digestion. Twenty micrograms of estrone increased cellulose digestion 63 percent ($P > 0.01$) and 20 micrograms of cholesterol increased it 25 percent ($P > 0.05$).

Comparative Values of Various Feedstuffs in Dairy Cattle Rations

A scarcity of information on the feeding value for lactating dairy cows of rations containing combinations of various grains and protein supplements undoubtedly can be attributed to the cost of such experiments. Because of the lack of data, a divergence of opinion has arisen regarding the value of certain protein-rich feeds which are used most widely in dairy cattle rations.

Ragsdale (1947), Holdaway, et al. (1925) and Nevins (1931) observed that soybean oil meal could be substituted for cottonseed meal in dairy cattle rations without affecting milk production. Nevins (1931) and Ragsdale (1947) reported that soybean oil meal and linseed meal are of equal value for maintenance and milk production, when the protein content of the rations is equal. Soybean oil meal gave slightly better results than linseed meal for milk production, when substituted pound for pound, according to Fairchild, et al. (1924). They found that somewhat less grain was required with soybean oil meal than when linseed meal was fed and concluded that, in general, soybean oil meal and linseed meal are nearly equal for milk or fat production.

When cottonseed meal and soybean oil meal were fed individually in equal amounts to lactating cows, Cook (1913) found that cottonseed meal produced 1.4 percent more milk but soybean oil meal produced 3.8 percent more butterfat. Cook (1913) concluded that soybean oil meal was worth slightly more than cottonseed meal.

Price (1908) and Lindsay, et al. (1924) reported that cottonseed meal had the same feeding value as ground soybeans.

Moore (1901) concluded that one pound of cottonseed meal is equal to one and one half pounds of wheat bran for dairy cows.

Michels (1910) reported that cottonseed meal produced slightly, but not significantly, better results with lactating dairy cattle than linseed meal.

McCandlish, et al. (1922) concluded that ground soybeans are worth 1/3 more than linseed meal for dairy cows. Olson (1925) reported that ground soybeans are 17.7 percent more efficient than old process linseed meal for butterfat production and 19.9 percent more efficient for milk production when alfalfa hay and corn silage served as roughages. Fairchild, et al. (1924), Nevins (1931) and Schafer (1927) found that ground soybeans gave better results with dairy cattle than soybean oil meal or linseed meal when substituted pound for pound. Nevins (1940) indicated that ground soybeans cannot be economically substituted for linseed meal if the roughage is mostly soybean hay. Ground soybeans were found by Crandall and Turk (1936) to furnish protein of excellent quality that balances the deficiencies of farm grains, and to equal linseed meal or cottonseed meal as a protein-rich feed for dairy cows. Cracked soybeans were also reported to be equal in value to soybean oil meal by Ely (1941). Monroe, et al. (1951) concluded that raw soybeans and soybean oil meal are of approximately equal feeding value for dairy cows under practical feeding conditions and can be fed up to 20 percent of the grain mixture without suppressing the carotene or vitamin A content of blood or milk. Cannon and Espe (1931) reported that cracked soybeans are just as efficient for dairy cows as is a mixture of soybeans, linseed meal and cottonseed meal. Nevins (1931) stated that single protein-rich feeds such as soybean oil meal and cottonseed meal in grain mixtures for dairy cattle gave just as good results as did mixtures of these feedstuffs.

It is generally concluded that the various protein supplements are of about equal value for dairy cows and " - the kind or quality of protein in rations for dairy cows is of little importance when the rations contain good roughage and are made up of feeds that are otherwise satisfactory," Morrison (1948), p. 710.

One or two farm grown grains were reported by Monroe and Krauss (1946a, 1946b) to be equal to complex mixtures for dairy cows, at least for short periods of time.

Associative Values of Various Feedstuffs for Dairy Cattle

Watson, et al. (1942) investigated any changes in the digestibility of individual feeds when incorporated into mixtures. From the values of the individual grains, a calculation was made of the coefficients of digestibility of the grain mixture. These calculated values were practically identical with the observed values determined by digestibility trials. These workers also concluded that there was no associative effect of digestibility among bran, gluten feed and soybean oil meal. Watson, et al. (1941) found no statistically significant differences between barley fed alone and barley fed in mixtures, but in the case of oats the values calculated from the mixed ration with hay were five percent lower in feeding value than the values obtained when oats were fed alone. On the contrary, Forbes, et al. (1943) indicated that there is true nutritive significance in the effects of feed combinations on the apparent digestibility. They concluded that " - combinations of feedstuffs affect apparent digestibility, not directly, but through the agency of alimentory microorganisms at the expense of feed nutrients and are then digested by the animal." Watson, et al. (1947a) reported that the digestibility of barley was the same when fed with timothy, when fed with alfalfa, or when fed alone to sheep or steers. Watson, et al. (1947b) found that, for rations containing a protein concentrate, a carbohydrate concentrate, and a roughage, the nutritive ratio does not influence the digestibility between the approximate range of 1:2 to 1:9.

APPARATUS, MATERIALS AND METHODS

The miniature artificial rumen and technique described by Radisson (1955) was modified for the purpose of these experiments (Plate I). As used in this study, the miniature artificial rumen consisted of dialysis tubing[1] 15/16 inches wide and about 12.5 cm. long. This tubing was tied

---

[1] Dialysis tubing No. 4463--A2 obtained from the Arthur H. Thomas Company, Philadelphia, Pennslyvania.

at one end with a string to form a sac into which the substrate and the rumen fluid, used as inoculum, were placed. The sac was then suspended in a four-ounce wide-mouth jar which contained approximately 100 ml. of McDougall's (1948) synthetic saliva.

The composition of this synthetic saliva is shown in Table 1. It was

Table 1. Composition of synthetic saliva.*

Constituent**	Quantity
$NaHCO_3$	9.8
$Na_2HPO_4 - 7H_2O$	6.5
HCl	0.57
NaCl	0.47
$CaCl_2.2H_2O$	0.05
$MgSO_4.7H_2O$	0.12
Distilled Water	1 liter

* McDougall (1948)

** All chemicals were reagent grade.

found that equally as good results could be obtained without bubbling carbon dioxide into this solution. The jar (Plate I) was closed with a No. 10 rubber stopper which contained a hole into which was inserted the open end of the fermentation sac. The sac was kept in place and open by inserting a glass tube about three cm. long and 16 mm. in diameter into the portion of the sac within the No. 10 rubber stopper. This glass tube was fitted with a No. 1 rubber stopper which contained a hole in which was inserted a capillary tube about three cm. long and seven mm. in diameter with a one mm. bore. A two inch length of 7/32 inch rubber tubing was placed on the capillary tube. A pinch clamp was then placed on the rubber tubing to permit the manual release of gas at various intervals during the fermentation period.

Due to apparent differences in air displacement, which developed after the rubber stoppers were installed, an escape of fermentation medium resulted each time the gas was released manually. Fitting the No. 10 rubber stopper with seven mm. capillary tubing prevented this loss of fermentation medium at the time of gas release.

Prior to each experiment, the substrates (prairie hay, individual grains, and filter paper) which were finely ground to pass through the fine screen of a Wiley mill, were weighed and placed in the dialysis sacs. Predetermined quantities of finely ground filter paper were added to the dialysis sacs in all but the first fermentation in order to equalize the initial amount of cellulose contained in each of the feedstuffs mixtures being studied. Additions of aureomycin were made only at the time of inoculation and proportionate quantities were placed in the salt solution outside the sac so that the concentrations were the same on both sides of the semi-permeable membrane. Since the quantity of aureomycin added at the start of the first fermentation did not produce as great a depression as was hoped for, larger quantities were added prior to subsequent fermentations.

Rumen samples were obtained (from fistulated calves) between 9 A.M. and 11 A.M. on the date of the incubation. The animals from which the rumen samples were obtained were two year old identical twin steers of mixed breeding, predominantly dairy. These twins were fed prairie hay ad libitum and four pounds per day of grain mix (18 percent crude protein) consisting of 400 pounds milo, 365 pounds wheat bran, 200 pounds soybean oil meal, 20 pounds salt and 10 pounds teamed bonemeal.

EXPLANATION OF PLATE I

Unassembled "miniature artificial rumen"

Glass insert -- Inserted into dialysis sac
(1 inch from upper end of sac)

Dialysis sac -- Inserted into No. 10 rubber
stopper
(Upper end of sac extends 1/4 inch above
rubber stopper)

No. 10 rubber stopper -- Inserted into 8 ounce
jar

Gas release mechanism -- Inserted into glass
insert

PLATE I


GAS RELEASE
MECHANISM
GLASS INSERT
NO. 10 RUBBER STOPPER
16 D
8 OUNCE JAR
DIALYSIS SAC

A washed suspension technique was described by Sijpesteijn and Elsden, (1952) which resulted in a more complete separation of the crude rumen liquor used in their artificial rumen studies. This separation involved centrifugations of rumen liquor at several speeds after each of which the resulting portions were fractionated. The technique was criticized by Sijpesteijn and Elsden for the following reasons:

1. It is questionable whether the composition of the washed suspension is quantitatively the same as the population of the rumen, even neglecting the removal of the protozoa by the preliminary centrifugation.

2. The fractionation is arbitrary and seldom sharp, and it was frequently observed that a considerable amount of the bacterial fraction other than protozoa is removed during the first brief centrifugation.

3. A significant proportion of the population is concerned with the fermentation of such insoluble materials as cellulose, hemicellulose, polyuronides, and starch, and it is probable that many of these organisms will be attached to the particulate matter removed during the preparation of the suspension.

4. It is possible that the more delicate organisms are inactivated during the preparation of the suspension. Thus, it is probable that the final suspension is deficient in the larger bacteria, in organisms attacking insoluble materials, and in the more delicate bacteria.

In view of these limitations of the washed suspension technique, crude rumen liquor was employed as inoculum throughout these experiments.

The rumen samples were squeezed through two thicknesses of cheese cloth, immediately after removal from the animal, and again just prior to the inoculation. An insulated container was used to transport the inoculum from the barn to the laboratory. This prevented any marked decrease in temperature of the rumen liquid. The period of time between the removal of the inoculum from the animal and the start of the incubation was usually less than one hour. Ten ml. of the inoculum were placed into each fermentation sac and the mixing of the inoculum and the substrate was obtained by gentle kneading of the sac, which then was immersed in the synthetic saliva. The jars (64 in each fermentation -- Plate II) were placed in a constant temperature water bath maintained at 39 ± 1° C. The contents of the fermentation sacs were mixed by removing the No. 10 rubber stopper and gently kneading the membrane, at the same time squeezing the pinch clamp to release the gas. This was repeated every two hours for the first 12 hours and then two or three times during the last 12 hours of incubation. Fitting the No. 10 rubber stopper with capillary tubing appeared to reduce the rate at which the pressure within the sac developed. Thus, the initial release of gas took place two hours after inoculation instead of 30 to 45 minutes, as recommended by Radisson (1955). After 24 hours of incubation, fermentation was stopped by the addition of approximately five ml. of 95 percent ethyl alcohol. The contents of each fermentation sac were removed with the aid of a stream of alcohol from a wash bottle, dried at 100° C., and analyzed for cellulose by the method of Crampton and Maynard (1938).

The above described in vitro technique was used to study the effect of several grains and protein supplements, with and without aureomycin, on the digestion of cellulose in prairie hay. Intentionally, prairie hay of poor quality was selected for this purpose. All grains and protein supplements used were, as far as could be determined, of local origin. The cellulose content of these feedstuffs mixtures (Table 2) was standardized by additions of finely ground filter paper (cellulose content of the mixtures used in the first in vitro digestion trial herein reported was not standardized). The protein content of these rations was standardized to 18 percent crude protein (calculated from Morrison, 1948, pp. 1086 - 1131) by adjusting the amount of protein supplement incorporated into the mixtures. A proportion of 500 mg. of prairie hay to 200 mg. of grain mixture

EXPLANATION OF PLATE II

Constant temperature water bath containing group of "miniature artificial rumens"

PLATE II

was used as substrate, upon the assumption that an average dairy cow weighing 1200 pounds and producing 30 pounds of milk per day would be fed a similar proportion of hay and grain (approximately 24 pounds of prairie hay and 10 pounds of an 18 percent grain mixture).

Table 2. Cellulose content of feedstuffs tested.*

Feedstuff**	Cellulose %
Corn	2.38
Oats	10.20
Milo	3.02
Barley	4.62
Wheat Bran	9.26
S O M	7.78
C M	10.16
L M	8.18
Raw Soybeans	6.66
Prairie Hay	31.86

* Determined by the method of Crampton and Maynard (1938).

** All feedstuffs were, as far as could be determined, of local origin.

Precision of this artificial rumen technique was ascertained by including, in duplicate, each of the mixtures being tested and determining the deviation from the mean of each of the duplicates. The averages of the duplicates appeared reliable since deviations were in most cases of a smaller magnitude than the differences between mixtures.

RESULTS

The experimental results herein reported were obtained from four trials with the artificial rumen. Each of these trials contained 16 different combinations of feedstuffs in duplicate without aureomycin, and the same combinations in duplicate with aureomycin. The first of the trials (Plate III and Appendix Table 1) was designed to test the effect on the percent of cellulose digested when combinations of corn, oats, milo or barley each in combination with either soybean oil meal, linseed meal or cottonseed meal were added to prairie hay in the artificial rumen.

The addition of 0.2 mcg. of aureomycin per ml. of rumen liquid generally depressed the digestion of cellulose with most of the combinations tested. However, a slight increase in cellulose digestion was obtained in the presence of certain combinations when aureomycin was included, but this increase occurred with few of the feedstuffs combinations. Also, these few resulting increases were more apparent than real since they were in no case greater than the differences between duplicates. The combinations of feedstuffs tested in this trial did not appear to supply all of the nutrients required by cellulolytic microorganisms to overcome the type of stress generally imposed by aureomycin.

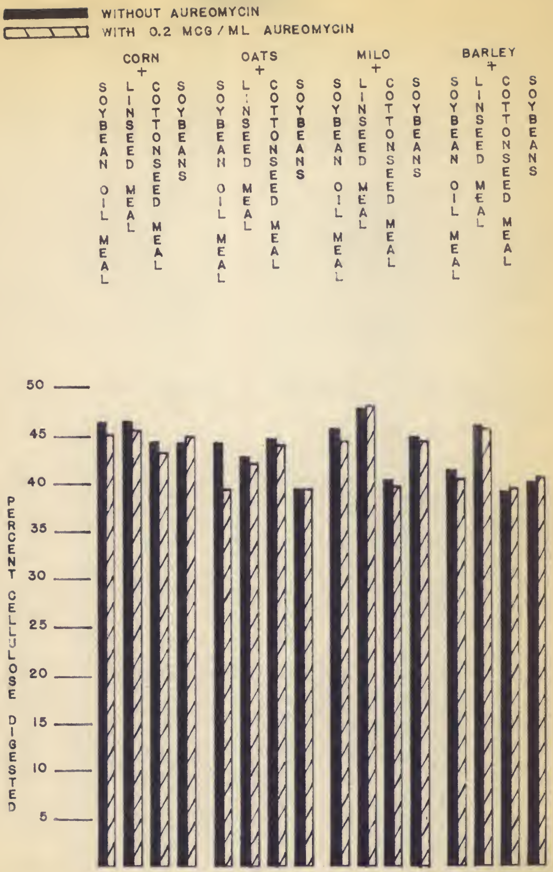
When used without aureomycin, the combinations in this trial containing corn had less fluctuation in the percent of cellulose digested than combinations containing any other cereal grain tested. Although cellulose digestion was occasionally higher with certain combinations of other cereal grains, corn appears to contain nutrients required by rumen microorganisms that will balance the deficiencies of the various protein-rich feeds more uniformly than oats, milo or barley.

The wide fluctuations in cellulose digestion which resulted when combinations of oats, milo or barley with certain protein-rich feeds were added to the artificial rumen indicates that certain feedstuffs combinations contain nutrients favorable to cellulolytic microorganisms. A lower percentage of cellulose digestion was obtained with the combination

EXPLANATION OF PLATE III

Micro-biological digestion of cellulose in prairie hay and either corn, oats, milo, or barley, each in combination with each of soybean oil meal, linseed meal, and cottonseed meal -- with and without aureomycin added to each combination.

PLATE III


WITHOUT AUREOMYCIN
WITH 0.2 MCG / ML AUREOMYCIN
CORN +
OATS +
MILO +
BARLEY +
SOYBEAN OIL MEAL
LINSEED MEAL
COTTONSEED MEAL
SOYBEANS
PERCENT CELLULOSE DIGESTED
50
45
40
35
30
25
20
15
10
5

of oats plus ground soybeans than with any of the other combinations of protein-rich feed and oats.

Cellulose digestion was also lower with the milo plus cottonseed meal combination than with milo plus any of the other protein-rich supplements.

When barley was combined with linseed meal, the percent of cellulose digested was higher than when barley was combined with any other protein-rich supplement used in this trial.

Similar fluctuations were apparent in cellulose digestion of mixtures resulting from combinations of the protein-rich feed with cereal grains. However, linseed meal, when combined with each of the cereal grains resulted in a generally greater micro-biological digestion of cellulose than any other single protein-rich supplement plus one of the single grains. Mixtures containing soybean oil meal ranked next in this respect.

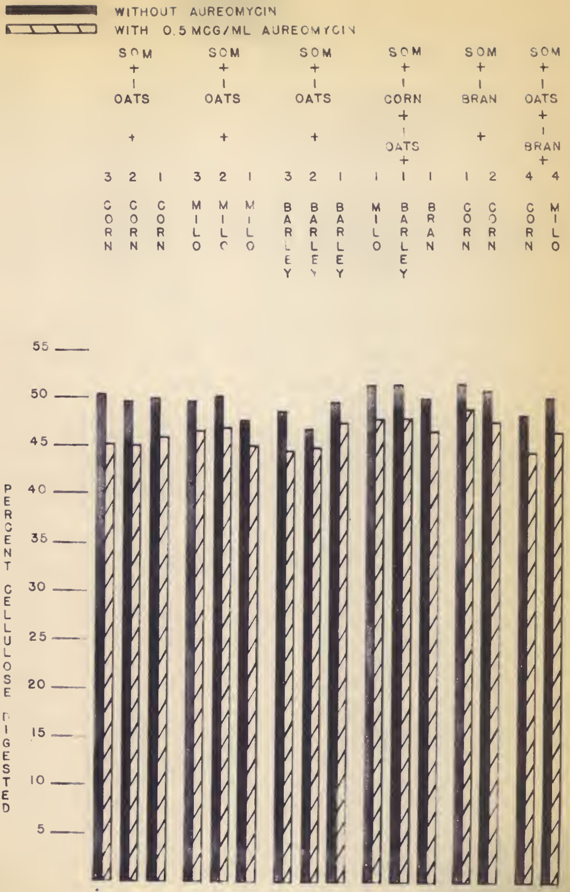
The results in trial 1 indicate that single cereal grains plus any of the protein-rich supplements possess different values for the micro-biological digestion of cellulose. This fact may be of importance to farmers who are feeding certain of these combinations. The trials were conducted under conditions simulating those which prevail within the bovine rumen, but tests were not made with animals. Therefore, application of the interpretations herein reported must be made with consideration of these limitations until the results are substantiated with further in vitro work, or with animal experiments.

Trial 2 (Plate IV and Appendix Table 2) was designed to test combinations of one protein-rich feed (soybean oil meal) plus two and three cereal grains (corn, oats, milo, barley and wheat bran) with prairie hay. In certain mixtures, the proportion of one cereal grain was varied to determine whether different quantities in a mixture would alter the percentage

EXPLANATION OF PLATE IV

Micro-bilogical digestion of cellulose in prairie hay and certain combinations of soybean oil meal, oats, corn, and wheat bran with certain proportions of corn, milo, barley, wheat bran -- with and without aureomycin added to each combination.

PLATE IV


WITHOUT AUREOMYCIN
WITH 0.5 MCG/ML AUREOMYCIN
SOM + 1 OATS + 3 CORN, 2 CORN, 1 CORN
SOM + 1 OATS + 3 MILO, 2 MILO, 1 MILO
SOM + 1 OATS + 3 BARLEY, 2 BARLEY, 1 BARLEY
SOM + 1 CORN + 1 OATS + 1 MILO, 1 BARLEY, 1 BRAN
SOM + 1 BRAN + 1 CORN, 2 CORN
SOM + 1 OATS + 1 BRAN + 4 CORN, 4 MILO
PERCENT CELLULOSE DIGESTED
55
50
45
40
35
30
25
20
15
10
5

of cellulose digestion. The concentration of aureomycin was increased to 0.5 mcg. per ml. of rumen liquid since 0.2 mcg. per ml. had not produced sufficient stress to distinguish differences between mixtures in offsetting any depressing affect of aureomycin.

When aureomycin was omitted from the mixture, combinations of two or more cereal grains plus soybean oil meal had less fluctuation, in percent of cellulose digested, than when single combinations of these feedstuffs were tested. Therefore, larger numbers of feedstuffs in the combinations tested, appear to supply a more adequate balance of nutrients required by the microorganisms which digest cellulose.

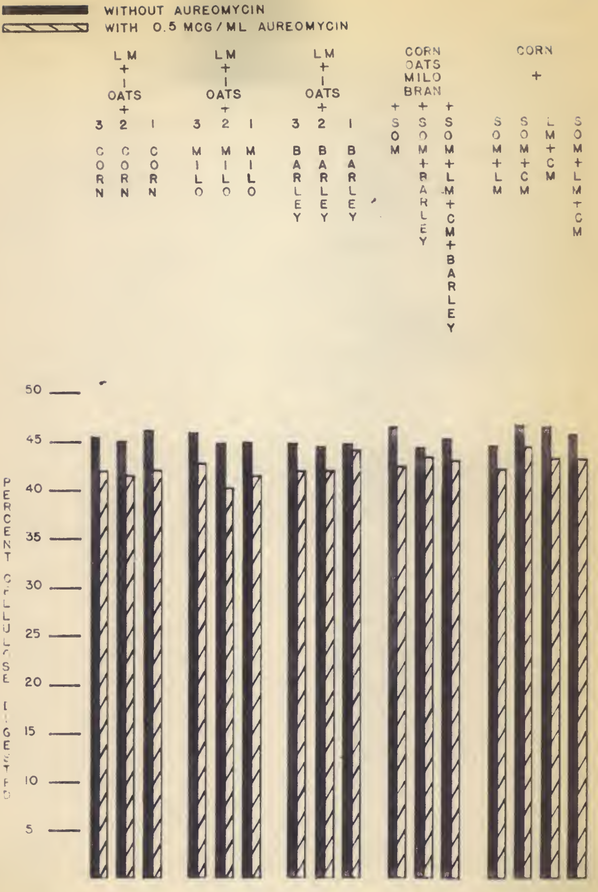
The increased quantity of aureomycin added caused a comparatively uniform reduction of the percent of cellulose digested in the presence of each of the combinations or proportions of ingredients tested. Therefore, provided the environment in which these feedstuffs were tested simulates that which occurs within the live animal, the combinations and proportions of feedstuffs tested in this trial might be assumed to be of equal value for micro-biological digestion of cellulose under the conditions of stress imposed by the aureomycin.

Altering the proportion of a cereal grain in each of the combinations tested had no consistent effect on the percent of cellulose digested. This substantiates the equality of these cereal grains in their influence on cellulose digestion when combined with other feeds.

In trial 3 (Plate V and Appendix Table 3), linseed meal plus oats and various proportions of corn, milo or barley with prairie hay were tested. Also, various combinations of protein-rich feeds (soybean oil meal, linseed meal and cottonseed meal) plus certain cereal grains (corn, oats, milo, barley and wheat bran) with prairie hay were tested. The aureomycin con-

EXPLANATION OF PLATE V

Micro-biological digestion of cellulose in prairie hay and linseed meal plus oats with certain proportions of corn, milo or barley; also in prairie hay and combinations of corn, oats, milo, wheat bran, soybean oil meal, linseed meal and cottonseed meal -- with and without aureomycin added to each combination.


WITHOUT AUREOMYCIN
WITH 0.5 MCG/ML AUREOMYCIN
LM + I OATS + 3 CORN
LM + I OATS + 2 CORN
LM + I OATS + 1 CORN
LM + I OATS + 3 MILO
LM + I OATS + 2 MILO
LM + I OATS + 1 MILO
LM + I OATS + 3 BARLEY
LM + I OATS + 2 BARLEY
LM + I OATS + 1 BARLEY
CORN OATS MILO BRAN
+ SOM
+ SOM + BARLEY
+ SOM + LM + CM + BARLEY
CORN +
SOM + LM
SOM + CM
LM + CM
SOM + LM + CM
PERCENT CELLULOSE DIGESTED
50
45
40
35
30
25
20
15
10
5

centration was the same as in trial 2.

The digestion of cellulose was uniform when combinations of linseed meal and certain cereal grains were tested in the artificial rumen. Increasing the proportion of a cereal grain in certain mixtures did not alter the percent of cellulose digested. These results substantiate the similarity of effects on micro-biological digestion of cellulose by various proportions of cereal grains mentioned previously when the combination of soybean oil meal and cereals were discussed.

Combinations of certain protein-rich feeds with various cereal grains are of similar value for micro-biological digestion of cellulose under the conditions of this experiment. This indicates that the protein-rich feeds herein tested can be interchanged without affecting the digestion of cellulose by rumen microorganisms in an artificial rumen.

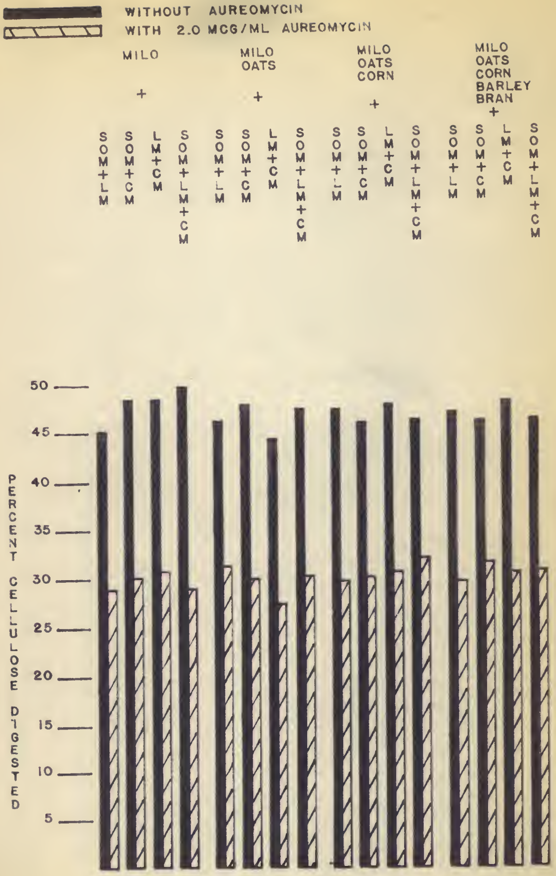
Trial 4 (Plate VI and Appendix Table 4) was designed to test the effect on percent of cellulose digested when other combinations of protein-rich feeds (soybean oil meal, linseed meal and cottonseed meal) each in combination with certain cereal grains (corn, oats, milo, barley, and wheat bran) were added to prairie hay in the artificial rumen. The quantity of aureomycin was increased to 2.0 mcg. per ml. to place a greater stress on the microorganisms and thereby detect the difference between certain grain mixtures in providing nutrients required by the microorganisms to withstand this condition.

The similarity in value found in trial 3 for the combinations of certain protein-rich feeds with various cereal grains tested was also found to apply to the mixtures tested in trial 4. Increasing the quantity of aureomycin consistently reduced the percent of cellulose digested in the presence of all of the combinations tested. This is further evidence of the equality of the combinations tested for cellulolytic activity.

EXPLANATION OF PLATE VI

Micro-biological digestion of cellulose in prairie hay and certain combinations of milo, oats, corn, barley and wheat bran with combinations of soybean oil meal, linseed meal and cottonseed meal -- with and without aureomycin added to each combination.

PLATE VI


WITHOUT AUREOMYCIN
WITH 2.0 MCG/ML AUREOMYCIN
MILO +
MILO OATS +
MILO OATS CORN +
MILO OATS CORN BARLEY BRAN +
SOM+LM
SOM+CM
LM+CM
SOM+LM+CM
PERCENT CELLULOSE DIGESTED
50
45
40
35
30
25
20
15
10
5

DISCUSSION

The equality of soybean oil meal and linseed meal as a part of a grain mixture for dairy cows, as reported by Nevins (1931) and Ragsdale (1947) appears to be true in regard to comparative value for micro-biological digestion of cellulose (Plate IV and V and Appendix Tables 2 and 3). However, the superiority which Fairchild and Wilbur (1924) reported for soybean oil meal, when compared with linseed meal, in dairy rations for milk production was not true for cellulose digestion in the mixtures containing three or more feedstuffs as herein listed.

Substituting cottonseed meal for soybean oil meal in the mixtures or testing mixtures of these protein-rich feeds (Plates V and VI and Appendix Tables 3 and 4), had little effect on the percent of cellulose digested, which supports the findings of Ragsdale (1947), Holdaway, et al. (1925), and Nevins (1931) who compared these feedstuffs in regard to their effect on milk production. However, the superiority of soybean oil meal to cottonseed meal for butterfat production found by Cook (1913) was not apparent when these feeds were compared for their effect on cellulose digestion (Plates V and VI and Appendix Tables 3 and 4).

Ragsdale (1947), having made allowance for the slightly lower protein content of linseed meal, found that it could be substituted for any of the other higher protein supplements in dairy rations without affecting milk production. This substitution also appears possible without lowering the percent of cellulose digested by rumen microorganisms (Plates V and VI and Appendix Tables 3 and 4).

The slightly better milk production that could be obtained from cottonseed meal compared to linseed meal, as reported by Michels (1910), cannot be related to their comparative value for cellulose digestion *in vitro* since a similar percent of cellulose was digested when these feeds were used in feed mixtures either as the only protein supplement or with another protein supplement.

Although ground soybeans were tested in various mixtures (Plate IV and Appendix Table 1), these mixtures lacked the complexity of those tested by other workers. Therefore, it is not possible to relate the results herein reported for ground soybean mixtures to the results reported by others.

The equally good milk production reported by Nevins (1931) which resulted from single protein supplements as compared to mixtures of them, corresponds to the results obtained when similar combinations were compared for micro-biological digestion of cellulose (Plates V and VI and Appendix Tables 3 and 4).

A statement by Morrison (1948), p. 710 that " - the kind or quality of protein in rations for dairy cows is of little importance when the rations contain good roughage and are made up of feeds that are otherwise satisfactory ......", appears to apply to rations containing poor quality roughage with combinations of three or more grains and protein supplement averaging 18 percent protein (Plates IV, V and VI and Appendix Tables 2, 3, and 4).

Simple grain mixtures containing one cereal grain and one protein-rich feed did not result in as consistently high percentages of cellulose digested (Plate III and Appendix Table 1) as found with more complex mixtures (Plates IV, V and VI and Appendix Tables 2, 3, and 4). This does not appear to correspond to the equality of simple grain mixtures when compared to complex mixtures for milk production as reported by Monroe and

Krauss (1946a, 1946b).

The associative effects of the various grain mixtures appears to be lessened as the number of grains in the mixture are increased after two cereals are included. This absence of associative effect with mixtures of three or more grains supports the findings of Watson, et al. (1941, 1942, 1947a), but contradicts the statement made by Forbes, et al. (1943) that " - combinations of feeding stuffs affect apparent digestibility, not directly, but through the agency of alimentary microorganisms which grow at the expense of food nutrients and are then digested by the animal. Thus, the effects of food combinations on apparent digestibility come to possess true nutritive significance."

The greater in vitro digestion of cellulose which Burrough, et al. (1950c) found when linseed meal and soybean oil meal were tested alone in the presence of some basic constituents of available nitrogen, complex mineral mixture and cow manure extract, was not evident (Plates IV, V, and VI and Appendix Tables 2, 3, and 4) when these protein-rich feeds were incorporated into grain mixtures without the above mentioned basic constituents.

Thus, the balance of nutrients that is present in grain mixtures, being more complete than the nutrients present in single grains, reduces the net effect of certain feedstuffs on rumen microorganisms. Therefore, certain feedstuffs would be expected to affect cellulose digestion less when added to mixtures than when tested alone or in mixtures containing fewer feedstuffs. Also Burroughs, et al. (1950c) used filter paper as a source of cellulose. The nutrients present in the prairie hay, used as a primary source of cellulose in the experiments herein reported, could not be supplied by filter paper, and thus the microorganisms would have to obtain their nutritive requirements from the grains only. Thus, in the tests herein reported the microorganisms did not need to depend exclusively on the grains for nutrients. These rations were more typical of rations fed to ruminants.

The depressent effect of aureomycin (Plates III, IV, V and VI and Appendix Tables 1, 2, 3 and 4) supports the findings of Radisson (1955), who reported that by increasing the concentration of aureomycin from 2.0 to 10.0 mcg. per ml. the relative effect on cellulose digestion was in the direction of depression.

SUMMARY AND CONCLUSIONS

Combinations and proportions of cereal grains and protein-rich feeds (all standardized to 18 percent protein) with prairie hay were tested for their effect on the rumen micro-biological digestion of cellulose both with and without aureomycin added.

Aureomycin, within the respective levels used, was found to depress cellulose digestion to a similar extent with all but a few of the mixtures tested. As the concentration of the antibiotic was increased, the digestion of cellulose decreased with all of the mixtures tested.

Simple mixtures of single cereal grains with single protein-rich feeds added to prairie hay were found to differ in percentage of cellulose digested. Mixtures containing corn with several single protein-rich feeds fluctuated less in the percent of cellulose digested than any other mixture of a single cereal with a single protein-rich supplement. When mixtures of certain feedstuffs containing three ingredients were used, the percentage of cellulose digested was as high as for mixtures containing a greater number of feedstuffs.

Certain feedstuffs included in mixtures of three or more ingredients appear to be interchangable, as measured by their effects on the percent of cellulose digested.

The proportion of certain cereal grains in the mixtures tested did not affect the percentage of cellulose digested.

ACKNOWLEDGMENT

The author wishes to express his sincere gratitude to Dr. E. E. Bartley for his guidance, timely suggestions and material assistance in the undertaking and completion of this investigation, and in the preparation of this manuscript.

Gratitude is likewise expressed to Professor F. W. Atkeson for his interest in this study and for his administrative assistance; and to Dr. T. J. Claydon and Dr. F. C. Fountaine for their advice and suggestions throughout this investigation.

The author is also indebted to Dr. D. B. Parrish for provision of some of the laboratory facilities used in this study.

The author gratefully acknowledges the generous supply of aureomycin supplied by Lederle Laboratories for use in this investigation.

LITERATURE CITED

Arias, Carlos, Wise Burroughs, P. Gerlaugh, and R. M. Bethke.
The influence of different amounts and sources of energy upon in vitro urea utilization by rumen microorganisms. J. Ani. Sci. 10:683. 1951.

Belasco, I. J.
New nitrogen feed compounds for ruminants -- A laboratory evaluation. J. Ani. Sci. 13:601-610. 1954.

Bentley, O. G., R. R. Johnson, S. Vanesko, and C. H. Hunt.
Studies on factors needed by rumen microorganisms for cellulose digestion in vitro. J. Ani. Sci. 13:581-593. 1954a.

Bentley, O. G., A. Lehmkuhl, R. R. Johnson, T. V. Hershberger, and A. L. Moxon.
The 'cellulolytic factor' activity of certain short chained fatty acids. J. Amer. Chem. Soc. 76:5000. 1954b.

Brooks, C. C., G. B. Garner, M. C. Muhrer, and W. H. Pfander.
Effect of some steroid compounds on ovine rumen function. Sci. 120: 455-456. 1954.

Burroughs, Wise, Carlos Arias, P. De Paul, P. Gerlaugh, and R. M. Bethke.
In vitro observations upon the nature of protein influences upon urea utilization by rumen microorganisms. J. Ani. Sci. 10:672. 1951a.

Burroughs, Wise, H. G. Headley, R. M. Bethke, and P. Gerlaugh.
Preliminary observations upon factors influencing cellulose digestion by rumen microorganisms. J. Nutr. 40:9-24. 1950a.

Burroughs, Wise, H. G. Headley, R. M. Bethke, and P. Gerlaugh.
Cellulose digestion in good and poor quality roughages using an artificial rumen. J. Ani. Sci. 9:513-522. 1950b.

Burroughs, Wise, A. Latona, P. De Paul, P. Gerlaugh, and R. M. Bethke.
Mineral influences upon urea utilization and cellulose digestion by rumen microorganisms using the artificial rumen technique. J. Ani. Sci. 10:693-705. 1951b.

Burroughs, Wise, J. Long, P. Gerlaugh, and R. M. Bethke.
Cellulose digestion by rumen microorganisms as influenced by cereal grains and protein-rich feeds commonly fed to cattle using an artificial rumen. J. Ani. Sci. 9:523-530. 1950c.

Cannon, C. Y., and D. L. Espe.
Cracked soybeans versus a mixture of cracked soybeans, linseed meal, and cottonseed meal as a source of protein for milk production. Iowa Agr. Exp. Sta. Ann. Report. 1931.

Cook, A. S.
Soybean meal versus cottonseed meal. N. J. Agr. Exp. Sta. Ann. Report. 1913.

Crampton, E. W., and L. A. Maynard.
The relation of cellulose and lignin content to the nutritive value of animal feed. J. Nutr. 15:383-395. 1938.

Crandall, W. T., and K. L. Turk.
Feeding the dairy cow efficiently. Cornell Ext. Bul. 363. Nov. 1936.

Ely, F.
Feeding dairy cows. Ky. Agr. Exp. Sta. Ext. Cir. 364. June 1941.

Fairchild, L. H., and J. W. Wilbur.
Soybean oil meal and ground soybeans as protein supplements in the dairy ration. Ind. Agr. Exp. Sta. Bul. 289. Dec. 1924.

Forbes, E. B., and R. W. Swift.
Conditions affecting the digestibility and the metabolizable energy of feeds for cattle. Penn. State College Bul. 452. 1943.

Gall, L. S., Wise Burroughs, P. Gerlaugh, and B. H. Edgington.
Rumen bacteria in cattle and sheep upon practical farm rations. J. Ani. Sci. 8:441-449. 1949.

Garner, G. B., M. E. Muhrer, W. C. Ellis, and W. H. Pfander.
Antibiotic-like substance and cellulose digestion stimulator found in fermented feeds and in rumen fluid. Sci. 435-436. 1954.

Holdaway, C. W., W. B. Ellett, and W. G. Harris.
The comparative value of peanut meal, cottonseed meal and soybean meal as sources of protein for milk production. Va. Agr. Exp. Sta. Tech. Bul. 28. Feb. 1925.

Huhtanen, C. N., R. K. Saunders, and L. S. Gall.
Fiber digestion using the miniature artificial rumen. J. Dairy Sci. 37:328-335. 1954.

Hungate, R. E.
The anaerobic mesophilic cellulolytic bacteria. Bact. Rev. 14:1. 1950.

Hunt, C. H., O. G. Bentley, T. V. Hershberger, and J. H. Cline.
The effect of carbohydrates and sulphur on B-vitamins synthesis, cellulose digestion, and urea utilization by rumen microorganisms in vitro. J. Ani. Sci. 13:570-580. 1954.

Lindsay, J. B., E. B. Holland, and P. H. Smith.
Effect of soybean meal and soybean oil upon composition of milk and butterfat. Mass. Agr. Exp. Sta. Report, Part 2. pp. 99-100. 1924.

Louw, J. G., H. H. Williams, and L. A. Maynard.
A new method for the study in vitro of rumen digestion. Sci. 110: 478-480. 1949.

Marston, H. R.
The fermentation of cellulose in vitro by organisms from the rumen of sheep. Biochem. J. 42:564-574. 1948.

McCandlish, A. C., E. Weaver, and L. A. Lunde.
Soybeans as a home-grown supplement for dairy cows. Iowa Agr. Exp. Sta. Bul. 204. Feb. 1922.

McDougall, E. I.
Studies on ruminant saliva: I. The composition and output of sheeps saliva. Biochem. J. 43:99-109. 1948.

Michels, R.
Feeding dairy cattle. N. C. Agr. Exp. Sta. Ann. Report. 1910.

Monroe, C. F., J. W. Hibbs, and R. G. Washburn.
Soybeans in dairy rations. Hoards Dairyman. 96:962. Dec. 1951.

Monroe, C. F., and W. E. Krauss.
Simple versus complex grain mixtures in dairy rations: I. Winter feeding of milking cows. Ohio Agr. Exp. Sta. Bimonthly Bul. 238. Jan.-Feb. 1946a.

Monroe, C. F., and W. E. Krauss.
Simple versus complex grain mixtures in dairy rations: II. Summer feeding to milking cows on pasture. Ohio Agr. Exp. Sta. Bimonthly Bul. 239. Mar.-Apr. 1946b.

Moore, J. S.
Feeding dairy cows. Miss. Agr. Exp. Sta. Bul. 70. Dec. 1901.

Morrison, F. B.
Feeds and feeding. 21st ed. Ithaca: Morrison Publishing Co. 1948.

Nevins, W. B.
Feeding the dairy herd. Ill. Agr. Exp. Sta. Cir. 372. June 1931.

Nevins, W. B.
Feeding the dairy herd. Ill. Agr. Exp. Sta. Cir. 502. Feb. 1940.

Olson, Thomas M.
Soybeans for dairy cows. S. D. Agr. Exp. Sta. Bul. 215. June 1925.

Price, J. N.
Comparison of soybeans with other dairy feeds. Tenn. Agr. Exp. Sta. Bul. 80. June 1908.

Radisson, J. J.
Mode of action of antibiotics in the nutrition of the dairy calf. Unpublished PhD. Thesis, Kansas State College, Manhattan, Kansas. 1955.

Ragsdale, A. C.
Rations for livestock and poultry. Mo. Agr. Exp. Sta. Cir. 319. Nov. 1947.

Sijpesteijn, A. K., and S. R. Elsden.
The metabolism of Succinic acid in the rumen of the sheep. J. Biochem. 52:41-45. 1952.

Schaefer, O. G.
Soybeans and soybean hay in the dairy ration. Minn. Agr. Exp. Sta. Bul. 239. Aug. 1927.

Wasserman, R. H., C. W. Duncan, E. S. Churchill, and C. F. Huffman.
The effect of antibiotics on in vitro cellulose digestion by rumen microorganisms. J. Dairy Sci. 35:571-580. 1952.

Watson, C. J., J. A. Campbell, W. M. Davidson, C. H. Robinson and G. W. Muir.
Digestibility studies with ruminants. VIII. Associative digestibility of hay and grains. Scientific Agr. 22:250-270. 1941.

Watson, C. J., J. W. Kennedy, W. M. Davidson, C. H. Robinson, and G. W. Muir.
Digestibility studies with ruminants. IX. Associative digestibility of grains: wheat bran, gluten feed and soybean oil meal. Scientific Agr. 22:561-570. 1942.

Watson, C. J., J. W. Kennedy, W. M. Davidson, C. H. Robinson, and G. W. Muir,
Digestibility studies with ruminants. X. Relative associative effects of the roughages, timothy and alfalfa with barley. Scientific Agr. 27:175-182. 1947a.

Watson, C. J., J. W. Kennedy, W. M. Davidson, C. H. Robinson, and G. W. Muir,
Digestibility studies with ruminants. XI. The effect of the nutritive ratio of a ration upon its digestibility by cattle. Scientific Agr. 27:600-608. 1947b.

APPENDIX

Table 1. Micro-biological digestion of cellulose in prairie hay and either corn, oats, milo, or barley, each in combination with each of soybean oil meal, linseed meal, and cottonseed meal -- with and without aureomycin added to each combination.

Aureomycin	Hay	Concentrates[1]								Cellulose			
	Prairie	Corn	Oats	Milo	Barley	SOM	LM	CM	Raw Soybeans	Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500	148.9				54.8				174.7	81.7	46.7	0.2
0.2	"	"				"				"	78.5	44.9	0.1
0	"		164.1			35.9				186.4	82.1	44.0	0.6
0.2	"		"			"				"	73.3	39.3	0.3
0	"			160.7		39.3				174.8	79.4	45.4	0.2
0.2	"			"		"				"	76.6	43.8	1.8
0	"				165.6	34.4				177.2	73.7	41.6	0.2
0.2	"				"	"				"	71.3	40.2	0.1
0	"	128.8					71.2			175.8	82.4	46.9	0.4
0.2	"	"					"			"	80.3	45.7	-
0	"		147.8				52.2			186.3	79.6	42.7	0.2
0.2	"		"				"			"	78.8	42.3	1.1
0	"			143.5			56.5			175.8	82.7	47.0	0.3
0.2	"			"			"			"	82.7	47.0	0.3
0	"				149.8		50.2			177.9	81.1	45.6	0.6
0.2	"				"		"			"	80.1	45.0	0.5
0	"	142.9						57.1		176.1	77.5	44.0	0.2
0.2	"	"						"		"	75.1	42.6	0.2
0	"		159.3					40.7		187.3	83.1	44.4	0.4
0.2	"		"					"		"	81.6	43.6	0.4
0	"			155.6				44.4		176.1	70.0	39.8	0.9
0.2	"			"				"		"	68.0	38.6	1.5
0	"				163.2			36.8		178.2	69.5	39.0	3.3
0.2	"				"			"		"	70.0	39.3	0.7

Table 1 (Concl).

Aureomycin	Hay	Concentrates[1]								Cellulose			
	Prairie	Corn	Oats	Milo	Barley	SOM	LM	CM	Raw Soybeans	Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500	135.8							64.2	174.4	77.0	44.2	1.1
0.2	"	"							"	"	78.2	44.8	0.4
0	"		153.7						46.3	185.7	73.2	39.4	0.5
0.2	"		"						"	"	73.2	39.4	0.8
0	"			149.6					50.4	174.8	77.5	44.3	0.7
0.2	"			"	157.9				"	"	75.7	43.3	0.3
0	"				"				42.1	177.0	77.8	44.0	0.4
0.2	"								"	"	78.1	44.1	0.1

1 The concentrate mixtures were calculated (Morrison 1948) to contain 18 percent crude protein.

Table 2. Micro-biological digestion of cellulose in prairie hay and certain combinations of soybean oil meal, oats, corn, and wheat bran with certain proportions of corn, milo, barley, and wheat bran - with and without aureomycin added to each combination.

Aureomycin	Hay	Concentrates[1]						Filter[2] Paper	Cellulose			
	Prairie	Corn	Oats	Milo	Barley	Bran	SOM		Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500	114.3	38.1				47.6	4.7	182.3	91.9	50.4	0.4
0.5	"	"	"				"	"	"	83.0	45.5	1.0
0	"	102.4	51.2				46.4	3.7	"	90.6	49.7	0.4
0.5	"	"	"				"	"	"	82.1	45.1	0.2
0	"	78.0	78.0				43.9	1.8	"	90.8	49.8	0.3
0.5	"	"	"				"	"	"	83.7	45.9	-
0	"		40.4	121.2			38.4	4.2	"	90.5	49.7	0.2
0.5	"		"	"			"	"	"	85.2	46.7	0.1
0	"		53.9	107.8			38.2	3.3	"	91.6	50.2	1.0
0.5	"		"	"			"	"	"	85.7	47.0	0.3
0	"		81.2	81.2			37.6	1.3	"	86.9	47.7	0.1
0.5	"		"	"			"	"	"	82.0	45.0	1.3
0	"		41.7		124.9		33.4	2.4	"	88.8	48.7	0.5
0.5	"		"		"		"	"	"	81.3	44.6	0.3
0	"		55.5		110.9		33.6	1.6	"	85.5	46.9	1.2
0.5	"		"		"		"	"	"	81.8	44.9	0.6
0	"		82.9		82.9		34.2	0.0	"	90.7	49.7	0.7
0.5	"		"		"		"	"	"	86.4	47.4	0.3
0	"	53.9	53.9	53.9			38.2	3.6	"	93.7	51.4	0.5
0.5	"	"	"	"			"	"	"	87.4	47.9	0.7
0	"	53.3	53.3		53.3		40.2	2.7	"	93.9	51.5	0.4
0.5	"	"	"		"		"	"	"	87.3	47.9	0.0

Table 2 (Concl.).

Aureomycin	Hay	Concentrates[1]						Filter[2] Paper	Cellulose			
	Prairie	Corn	Oats	Milo	Barley	Bran	SOM		Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500	55.5	55.5			55.5	33.4	0.0	182.3	91.0	49.9	0.1
0.5	"	"	"			"	"	"	"	85.1	46.7	1.6
0	"	83.9				83.9	32.2	2.7	"	93.8	51.5	0.1
0.5	"	"				"	"	"	"	89.6	49.1	1.2
0	"	107.4				53.7	39.0	4.4	"	92.7	50.9	0.8
0.5	"	"				"	"	"	"	86.9	47.7	0.4
0	"	104.8	26.2			26.2	42.8	4.1	"	88.0	48.3	0.1
0.5	"	"	"			"	"	"	"	80.9	44.4	0.4
0	"		27.6	110.6		27.6	34.2	3.6	"	91.5	50.2	0.4
0.5	"		"	"		"	"	"	"	85.0	46.6	1.6

1 The concentrate mixtures were calculated (Morrison 1948) to contain 18 percent crude protein.

2 Filter paper was added to equalize the cellulose content of the concentrate mix.

Table 3. Micro-biological digestion of cellulose in prairie hay and linseed meal plus oats with certain proportions of corn, milo or barley; also in prairie hay and combinations of corn, oats, milo, wheat bran, soybean oil meal, linseed meal and cottonseed meal -- with and without aureomycin added to each combination.

Aureomycin	Hay	Concentrates[1]								Filter[2] Paper	Cellulose			
	Prairie	Corn	Oats	Milo	Barley	Bran	SOM	LM	CM		Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500	99.8	33.3					66.9		3.8	183.3	83.8	45.7	0.1
0.5	"	"	"					"		"	"	77.3	42.2	0.2
0	"	89.7	44.8					65.5		2.9	"	83.1	45.3	1.0
0.5	"	"	"					"		"	"	76.5	41.7	0.6
0	"	68.8	68.8					62.4		1.2	"	85.0	46.4	1.9
0.5	"	"	"					"		"	"	77.7	42.4	0.7
0	"		36.1	108.4				55.4		3.9	"	84.9	46.3	0.0
0.5	"		"	"				"		"	"	78.9	43.0	0.3
0	"		48.3	96.6				55.1		2.6	"	82.5	45.0	1.7
0.5	"		"	"				"		"	"	74.0	40.4	0.8
0	"		72.8	72.8				54.4		0.9	"	82.7	45.2	0.5
0.5	"		"	"				"		"	"	76.3	41.7	0.2
0	"		37.8		113.3			48.9		1.9	"	82.6	45.1	0.8
0.5	"		"		"			"		"	"	77.2	42.2	0.1
0	"		50.3		100.6			49.1		1.2	"	81.6	44.5	1.7
0.5	"		"		"			"		"	"	77.2	42.1	2.1
0	"		75.1		75.1			49.9		0.0	"	82.2	44.8	1.2
0.5	"		"		"			"		"	"	79.9	43.6	0.1
0	"	41.3	41.3	41.3		41.3	34.9			2.0	"	85.6	46.7	0.8
0.5	"	"	"	"		"	"			"	"	77.7	42.4	0.6

Table 3 (Concl.).

Aureomycin	Hay	Concentrates[1]								Filter[2] Paper	Cellulose			
	Prairie	Corn	Oats	Milo	Barley	Bran	SOM	LM	CM		Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500	33.1	33.1	33.1	33.1	33.1	34.4			2.5	183.3	81.6	44.5	1.1
0.5	"	"	"	"	"	"	"			"	"	79.7	43.5	0.1
0	"	32.0	32.0	32.0	32.0	32.0	13.4	13.4	13.4	2.1	"	83.2	45.4	0.5
0.5	"	"	"	"	"	"	"	"	"	"	"	79.2	43.2	0.7
0	"	140.5					29.7	29.7		6.9	"	81.9	44.7	1.7
0.5	"	"					"	"		"	"	77.4	42.2	0.1
0	"	146.0					27.0		27.0	6.7	"	85.8	46.8	0.0
0.5	"	"					"		"	"	"	81.4	44.4	-
0	"	136.6						31.7	31.7	5.9	"	85.2	46.5	0.7
0.5	"	"						"	"	"	"	79.1	43.2	0.2
0	"	140.4						19.9	19.9	19.9	"	83.8	45.7	1.3
0.5	"	"						"	"	"	"	79.2	43.2	0.1

1 The concentrate mixtures were calculated (Morrison 1948) to contain 18 percent crude protein.

2 Filter paper was added to equalize the cellulose content of the concentrate mix.

Table 4. Micro-biological digestion of cellulose in prairie hay and certain combinations of milo, oats, corn, barley and wheat bran with combinations of soybean oil meal, linseed meal and cottonseed meal -- with and without aureomycin added to each combination.

Aureomycin	Hay	Concentrates[1]								Filter[2] Paper	Cellulose			
	Prairie	Corn	Oats	Milo	Barley	Bran	SOM	LM	CM		Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500			153.6			23.2	23.2		6.7	178.4	80.5	45.1	0.3
2.0	"			"			"	"		"	"	51.6	28.9	0.2
0	"			158.4			20.8		20.8	6.5	"	86.6	48.5	0.3
2.0	"			"			"		"	"	"	53.8	30.2	0.2
0	"			150.4				24.8	24.8	5.9	"	86.9	48.7	0.1
2.0	"			"				"	"	"	"	55.0	30.8	0.3
0	"			154.3			15.3	15.3	15.3	6.4	"	88.9	49.9	0.7
2.0	"			"			"	"	"	"	"	51.6	28.9	0.8
0	"		77.9	77.9			22.1	22.1		1.2	"	83.3	46.7	1.5
2.0	"		"	"			"	"		"	"	55.9	31.3	0.5
0	"		80.2	80.2			19.8		19.8	0.9	"	85.3	47.8	1.5
2.0	"		"	"			"		"	"	"	53.3	29.9	0.3
0	"		76.3	76.3				23.7	23.7	0.6	"	78.9	44.3	0.7
2.0	"		"	"				"	"	"	"	48.8	27.4	0.1
0	"		78.2	78.2			14.5	14.5	14.5	0.9	"	84.7	47.5	0.0
2.0	"		"	"			"	"	"	"	"	53.8	30.2	0.1
0	"	50.0	50.0	50.0			25.0	25.0		3.2	"	84.5	47.3	0.6
2.0	"	"	"	"			"	"		"	"	52.6	29.5	0.7
0	"	51.7	51.7	51.7			22.5		22.5	2.9	"	82.2	46.1	0.6
2.0	"	"	"	"			"		"	"	"	53.1	29.8	1.0
0	"	48.9	48.9	48.9				26.7	26.7	2.5	"	85.2	47.8	1.2
2.0	"	"	"	"				"	"	"	"	54.8	30.7	0.9

Table 4 (Concl.).

Aureomycin	Hay	Concentrates[1]								Filter[2] Paper	Cellulose			
	Prairie	Corn	Oats	Milo	Barley	Bran	SOM	LM	GM		Initial Amount	Digested		Deviations from mean
mcg/ml	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	%	% ±
0	500	50.2	50.2	50.2			16.4	16.4	16.4	2.9	178.4	82.6	46.3	0.1
2.0	"	"	"	"			"	"	"	"	"	57.1	32.0	0.5
0	"	31.8	31.8	31.8	31.8	31.8	20.4	20.4		2.4	"	84.1	47.2	0.2
2.0	"	"	"	"	"	"	"	"		"	"	52.7	29.5	0.4
0	"	32.7	32.7	32.7	32.7	32.7	18.3		18.3	2.1	"	82.3	46.1	0.5
2.0	"	"	"	"	"	"	"		"	"	"	56.0	31.4	0.9
0	"	31.2	31.2	31.2	31.2	31.2		21.9	21.9	1.8	"	85.8	48.1	0.2
2.0	"	"	"	"	"	"		"	"	"	"	54.6	30.6	1.0
0	"	32.0	32.0	32.0	32.0	32.0	13.4	13.4	13.4	2.1	"	82.6	46.3	0.7
2.0	"	"	"	"	"	"	"	"	"	"	"	54.9	30.8	0.8

1 The concentrate mixtures were calculated (Morrison 1948) to contain 18 percent crude protein.

2 Filter paper was added to equalize cellulose content of the concentrate mix.

EFFECT OF VARIOUS COMBINATIONS AND PROPORTIONS OF FEEDSTUFFS WITH AND WITHOUT AUREOMYCIN ON THE IN VITRO DIGESTION OF CELLULOSE BY RUMEN MICROORGANISMS

by

Frank John Hanold

B. S., Kansas State College
of Agriculture and Applied Science, 1952

- - - - - - - - - -

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

There is a paucity of information on the influence of combinations of feedstuffs as commonly used in practical farm rations on the *in vitro* digestion of cellulose. The work herein reported was initiated for the purpose of furnishing more information on this subject. Various combinations of some commonly used feedstuffs were studied for their effect on the *in vitro* digestion of cellulose by rumen microorganisms. A slight improvement in the efficiency of utilization of the tremendous quantities of poor quality roughage available for livestock feeding would be of considerable economic importance.

A series of "miniature artificial rumens", each of which consisted of dialysis tubing 15/16 inches wide and about 12.5 cm. long, were used in these experiments. The tubing was tied at one end with a string to form a sac into which the substrate and the strained rumen fluid, used as inoculum, were placed. The sac was then suspended in a four-ounce wide-mouth jar which contained approximately 100 ml. of synthetic saliva.

Prior to each experiment, the substrates (prairie hay, individual grains, and filter paper), which were finely ground, were weighed and placed in the dialysis sacs. Each of the mixtures was tested with and without aureomycin, since this antibiotic has been shown to increase the nutrient requirements of rumen microorganisms as demonstrated by a decrease in digestion of cellulose in the presence of this antibiotic.

After about 30 hours of incubation, the contents of each artificial rumen were analyzed for cellulose and the amount digested was considered to be the difference between that which was found after incubation and that which was present in control samples of feed before incubation.

Aureomycin within the respective levels used was found to depress cellulose digestion to a similar extent with all but a few of the mixtures tested. As the concentration of the antibiotic was increased, the digestion of cellulose decreased with all of the mixtures tested.

Simple mixtures of single cereal grains with single protein-rich feeds added to prairie hay were found to differ in percentage of cellulose digested. Mixtures containing corn with several single protein-rich feeds fluctuated less in the percent of cellulose digested than any other mixture of a single cereal with a single protein-rich supplement. When mixtures of certain feedstuffs containing three ingredients were used the percentage of cellulose digested was as high as for mixtures containing a greater number of feedstuffs.

Certain feedstuffs included in mixtures of three or more ingredients appear to be interchangable, as measured by their effects of the percent of cellulose digested.

The proportion of certain cereal grains in the mixtures tested did not affect the percentage of cellulose digested.