UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH SERVICE
WASHINGTON, D.C. 20250

February 7, 1968

To : Participants in Ninth Conference on Rumen Function

From : C. R. Richards, Chairman of Conference, Cooperative State Research Service, U. S. Department of Agriculture, Washington, D. C.

Subject: Report on Ninth Conference on Rumen Function

A list of the participants in the conference is attached to the report which consists of copies of the abstracts of papers presented at the conference held at the Midland Hotel, Chicago, Illinois on November 29 and 30, 1967.

The conference convened at 9:00 a.m. on November 29 and was concluded about 12:00 Noon, November 30.

The program was organized by the chairmen of the respective panels. Dr. R. F. Barnes substituted for Dr. G. E. Carlson in conducting the Agronomic Panel. On behalf of the Conference I commend the chairmen for the organization and those who presented papers for the excellent reports made. The lively discussions illustrated the interest of those who attended.

The participants requested that a similar conference be held in 1969 at about the same time of year and at the Midland Hotel. A meeting room at the Midland Hotel has been reserved for Wednesday and Thursday, December 3 and 4, 1969.

Dr. E. E. Bartley was named chairman of a committee to investigate the pros and cons of formalizing this group into a national organization. He will choose others to serve with him.

I was asked to continue as chairman of the conference. A program committee was named which consists of the panel chairmen, R. W. Dougherty, A. D. McGilliard, G. E. Carlson and M. P. Bryant (replacing C. K. Smith, as chairman of the Microbiology Panel).

NINTH CONFERENCE ON RUMEN FUNCTION
held at
Midland Hotel, Chicago, Illinois
November 29-30, 1967

The following persons were in attendance during the two-day meeting:

NAME	ORGANIZATION
Allison, M. J.	National Animal Disease Laboratory, Ames, Iowa 50010
Barnes, R. F.	USDA, ARS. Department of Agronomy, Purdue University, Lafayette, Indiana 47907
Bartley, E. E.	Call Hall, Kansas State University, Manhattan, Kansas 66504
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Blake, J. T.	Department of Veterinary Science, Utah State University, Logan, Utah 84321
Bryant, M. P.	Department of Dairy Science, University of Illinois, Urbana, Illinois 61803
Carlson, I. T.	Agronomy Department, Iowa State University, Ames, Iowa 50010
Clark, J. G.	681 Col. Dewees Rd., Wayne, Pennsylvania 19087
Cook, R. M.	Dairy Department, Michigan State University, East Lansing, Michigan 48823
Crump, M. H.	Department of Physiology, Iowa State University, Ames, Iowa, 50010
Dehority, B. A.	Ohio Agricultural Research & Development Center, Wooster, Ohio 44691
Dobson, A.	Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, N. Y. 14850
Dougherty, R. W.	National Animal Disease Laboratory, Ames, Iowa 50010
Durbin, C. G.	Food & Drug Laboratory, Agricultural Research Center, Beltsville, Maryland 20705
Dziuk, H. E.	Department of Veterinary Physiology, University of Minnesota, St. Paul, Minnesota 55101
Elliott, F. C.	Crop Science Department, Michigan State University, East Lansing, Michigan 48823
Emery, R. S.	Dairy Department, Michigan State University, East Lansing, Michigan 48823
Fina, L. R.	Division of Biology-Microbiology, Kansas State University, Manhattan, Kansas 66504
Fulghum, R. S.	Department of Bacteriology, North Dakota State University, Fargo, North Dakota 58103
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Gessert, R. A.	Upjohn International, Kalamazoo, Michigan 49003

NAME	ORGANIZATION
Gillette, D. D.	Veterinary Physiology, Iowa State University, Ames, Iowa 50010
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Hargus, W. A.	International Mineral and Chemical Corporation, Libertyville, Illinois 60048
Hartmann, P. E.	Animal Biology, New Bolton Center, Kennett Square, Pennsylvania 19348
Hemken, R. W.	Dairy Science Department, University of Maryland, College Park, Maryland 20742
Houpt, T. R.	School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104
Huber, J. T.	Dairy Department, Michigan State University, East Lansing, Michigan 48823
Hull, M. W.	Veterinary Research Laboratory, Montana State University, Bozeman, Montana 59715
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Ingraham, R. H.	Veterinary Physiology, Iowa State University, Ames, Iowa 50010
Jacobson, D. R.	Department of Animal Science, University of Kentucky, Lexington, Kentucky 40506
Jinkins, J. W.	Box 5, Montfort, Wisconsin 53569
Johnson, R. J.	Animal Science Department, Washington State University, Pullman, Washington 99163
Kronfeld, D. S.	Animal Biology, New Bolton Center, Kennett Square, Pennsylvania 19348
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Manns, J. G.	Department of Veterinary Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
McGilliard, A. D.	Department of Animal Science, Iowa State University, Ames, Iowa 50010
McKinley, R. E.	75 Oak Ridge Rd., Verona, New Jersey 07044
Milligan, L. P.	Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada.
Mitchell, Jr.,G. E.	Animal Sciences Department, University of Kentucky, Lexington, Kentucky 40506
Oliver, T. J.	Microbiological Chemistry, Abbott Laboratories, North Chicago, Illinois 60064
Palmquist, D. L.	Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691
Purser, D. B.	Animal Husbandry, Michigan State University, East Lansing, Michigan 48823
Reid, R. L.	Animal Science Department, West Virginia University, Morgantown, West Virginia 26506
Reschly, L. J.	Department of Veterinary Physiology, University of Missouri, Columbia, Missouri 65201

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Richards, C. R.	CSRS, USDA, Rm. 444-A, Washington, D. C. 20250
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Sellers, A. F.	Department of Physiology, New York State Veterinary College, Ithaca, New York 14850
Smith, C. K.	Department of Veterinary Science, Ohio Agricultural Research & Development Center, Wooster, Ohio 44691
Smith, I. D.	Biochemistry Department, Abbott Laboratories, North Chicago, Illinois 60064
Swenson, M. J.	Department of Physiology & Pharmacology, Iowa State University, Ames, Iowa 50010
Theodorides, V. J.	SK & F Laboratories, 1522 Spring Garden Street, Philadelphia, Pennsylvania 19130
Thomas, J. W.	Dairy Department, Michigan State University, East Lansing, Michigan 48823
Thorlacius, S. O.	Department of Veterinary Physiology, Cornell University, Ithaca, New York 14850

REPORT ON
CONFERENCE ON RUMEN FUNCTION
held at
Midland Hotel, Chicago, Illinois
November 29 30, 1967

For the purpose of discussion, the program was divided into four panels. The identity of the panels and the chairman of each was as follows:

(a)	Physiopathology	R. W. Dougherty
(b)	Agronomic	G. E. Carlson
(c)	Physiology	A. D. McGilliard
(d)	Microbiology	C. K. Smith

PHYSIOPATHOLOGY PANEL

Using Herd Records to Isolate Environmental Effects Upon Milk Production - D. Dale Gillette and R. H. Ingraham, Iowa State University, Ames.

An adjusted daily milk production is obtained by the use of index numbers for the daily average cow quality and the daily average position in the lactation curve. The number of cows milking, as given by the herd book, are checked against a day by day tally from calfing through the stated duration of lactation. This adjusted milk production is plotted as contours on a plot of the temperature and humidity intersections.

Ruminal Urease and the Transfer of Urea-Nitrogen Across the Rumen Wall - T. Richard Houpt, University of Pennsylvania, Philadelphia.

The movement of urea-N across the wall of Pavlov-type rumen pouches was measured in goats and sheep as the concentration difference between blood plasma and pouch solutions was varied. When ruminal urease activity was reduced by repeated rinsing of the pouch, or by treatment with antibacterial agents, most urea transferred from the blood plasma appeared in saline solutions within the pouch as urea unchanged. Under these conditions, transfer of urea in either direction was linearly correlated with urea concentration difference, and extrapolation to zero concentration difference indicated that transfer would cease at that point. When ruminal urease activity was not disturbed, essentially all urea-N transferred from the blood plasma appeared in pouch solutions as ammonia-N. Furthermore, at normal blood urea concentrations, up to 13 times as much nitrogen was transferred into the pouch. It appears that urea per se moves through the rumen wall by simple diffusion. However, these results suggest the hypothesis that, under normal conditions, blood urea is hydrolyzed as it passes through the rumen wall before all epithelial diffusion barriers are crossed. The resultant ammonia - being of smaller and more lipid-soluble molecules - then diffuses more rapidly than would urea through the remaining epithelial layers. The overall effect is to enhance transfer of urea-N from blood into the rumen where it may be utilized in protein synthesis.

Blood Content of Rumen Papilla of the Cow - S. O. Thorlacius, New York State Veterinary College, Cornell University, Ithaca.

The blood content of a rumen papilla was estimated by extraction of the biopsied papilla with .01 M disodium ethylenediamine tetraacetate pH 10.9 and measuring change in optical density at 420 mμ following the addition of carbon monoxide. Application of acetic, butyric, carbonic and propionic acids increased the amount of blood per mg dry weight. This effect appeared to be localized to the area of application. (Supported by NIH Grant No. AM 04679)

The Absorption of Water from the Temporarily Isolated Ventral Sac of the Cow - A. Dobson and A. F. Sellers, New York State Veterinary College, Cornell University, Ithaca.

This new animal preparation allows the precise measurement of absorption from a defined part of the rumen of a conscious cow. The provision of a large fistula into the dorsal sac is the sole necessary surgery. The area of rumen under study is well isolated during experimental observations, but at all other times is undergoing its normal functions.

Water absorption from the ventral sac was observed by following changes in concentration of chromium ethylenediamine tetracetic acid, a nonabsorbed volume marker. When the osmotic pressure within the ventral sac was varied from 0.2 - 0.4 osmole/kg, the rate of water absorption bore a linear relation to the difference in osmotic pressure across the rumen epithelium. With sodium chloride or acetate under nitrogen in the rumen, no net water flux was observed when the osmotic pressure was close to the plasma. However, under more physiological circumstances a net water flux from rumen to blood against the activity gradient of water was observed. (Supported by NIH Grant No. AM 04679)

The Relationship of Environmentally Induced Rectal Temperatures to Feed Consumption and Milk Production in Holstein Cows - Rodney H. Ingraham and D. Dale Gillette, Iowa State University, Ames.

A modern dairy of 600 Holsteins at Culiacan, Mexico was experiencing large drops in fertility and production during the summer. One hundred unbred cows in early lactation were split into four pens in an experiment to determine the extent to which the summer weather was affecting them. Two pens served as controls. Two pens were used to determine the response of the cows to various means of cooling.

Air temperature and humidity in the corrals was monitored. Rectal temperatures were taken four times a day on 50 cows and two times a day on the other 50. Milk from each cow was weighed at milking time. An average of 37.4 pounds of concentrate and 22 pounds of alfalfa hay per cow was fed each day. Each morning uneaten feed was weighed and subtracted from the amount fed to determine feed consumption. Cows were fed by hand seven times a day to be sure hay and

grain were available at all times. The concentrate was 40% milo, 25% rice bran, 15% mollasis, and 20% premix.

At the start of the experiment, average daily ambient temperature was 81°F with an average relative humidity of 67% (THI 76.7). Daily average rectal temperature was 102.7°F. The day causing maximum rectal temperature response in the cows had an average ambient temperature of 88°F, RH 62% (THI 80.6). Maximum temperature for the day was 98°F. A pen of 25 cows responded with an average rectal temperature of 104.7°F for that day. Pen average of an early afternoon reading was 105.5°F. Individual rectal temperatures of 107°F were not uncommon.

Consumption of TDN on a given day was inversely correlated with rectal temperature on that day. The high level of grain fed was consumed as long as daily average rectal temperatures remained under 103.5°F. This is also the rectal temperature at which milk production dropped drastically. The extreme weather caused an average drop in daily milk from 53 pounds to 43 pounds.

There was a large difference in the rectal temperature response of individual Holstein cows to increased ambient temperatures and humidities. Standard deviation for average rectal temperatures of a pen of 25 cows was .4 in the milder weather at the beginning of the experiment and 2.4 at the more extreme weather given.

The adverse weather tended to be the result of increased minimum temperatures accompanied by increased humidities. With minimum ambient temperatures above 80°F and humidities above 60% cows were unable to loose sufficient heat at night and showed heat stress most strongly.

Some Tissue Enzyme Adaptations to Ruminal Fermentation - D. S. Kronfeld, University of Pennsylvania, Kennett Square.

The enzymic machinery of certain metabolically important tissues in ruminants is adapted to the effects of ruminal fermentation on the availability of nutrients such as acetate and glucose. The typical pattern of this set of adaptations may be determined by comparing various enzyme parameters found in adult ruminants with data from young ruminants or from monogasters. The most common data available at present are maximal activities of hexokinases, several dehydrogenases and acyl CoA synthetases. Data are also available on the affinities of hexokinases for glucose and of synthetases for acetate, propionate and butyrate. An attempt will be made to relate some published and unpublished enzymic data to the overall pattern of ruminant metabolism.

Venous and Ruminal Changes of FVA's and NH_3 - John W. Jinkins and Malcolm H. Crump, Iowa State University, Ames.

Chronic cannulas were implanted in the right ruminal vein of sheep. The method used was that of expanding silastic tubing with xylene and inserting polyvinyl tubing into the expanded end. The silastic portion was inserted into the vein about six inches. The polyvinyl end was exteriorized. This technique has produced very satisfactory results.

Venous blood samples and ruminal fluid samples were collected from sheep fed fresh alfalfa and birdsfoot trefoil. Urea and poloxalene were used as subtreatments. The blood samples were collected before feeding and each hour after feeding for five hours. They were analyzed for total volatile fatty acids and ammonia. Total volatile fatty acid concentration has ranged from 300 to 3925 micromoles per 100 cc of blood.

Ruminal fluid samples were collected before feeding and every one and one-half hours after feeding for three times. They were analyzed for total volatile fatty acids and total volatile bases. Concentrations varied depending upon the feeding regime, subtreatments and time. Total volatile base concentration ranged from 10 to 114 millimole per liter and total volatile fatty acid concentration ranged from 27 to 148 millimoles per liter.

AGRONOMIC PANEL

Bioassays for Nutritional Efficiency of Individual Alfalfa Plants - Fred C. Elliott, Michigan State University, East Lansing.

Bioassays assess simultaneously both positive and negative factors affecting nutritional efficiency. They must be adapted to individual cross-fertilizing plants since control of heredity is exercised at this level. Large numbers of plants are required for improvement through breeding and only small amounts of forage are available for bioassays.

Three bioassays have been used so far with alfalfa: Weanling growth responses in meadow voles (Microtus pennsylvanicus), a modified 6 hour in vitro rumen fermentation test for dry matter disappearance, and a Trichoderma assay for saponin levels. The results of these assays are not highly correlated but individual plants with high or low responses in all three have been found in a number of varieties. After two cycles of selection progress has been made and seed from the first cycle of improvement is available for comparative tests. Better bioassays are sought as guides to selection in alfalfa for leaf fractions where indicators of protein quantity, quality, and absence of antimetabolites to humans and non-ruminants are needed. For stem and residual fractions from leaf extraction bioassays indicating ruminant digestibility and efficiency would be most helpful.

Mineral Nutrition of Plants in Relation to Animal Performance - R. L. Reid and G. A. Jung, West Virginia University, Morgantown.

Marked deficiencies of minerals in the soil or diet have been related classically to clinical deficiency syndromes in domestic livestock and in man. Studies with ruminant animals indicate that mineral fertilization of forages may also result in a variety of effects relating to intake, digestion, palatability and utilization of the plant.

Initial trials with grass hay fertilized with phosphorus, potassium or different levels of nitrogen showed that, while treatment had little effect on energy digestibility or on intake, it had a marked effect on the relative palatability of the forage. Preference for P fertilized grass was associated with an altered plant mineral and carbohydrate composition and with a low level of P nutrition in the animal. Attempts to demonstrate a mechanism of "nutritional compensation" by studying the feeding behavior of P-deficient and P-adequate sheep were negative.

For sheep fed grass hays or fresh herbage in conventional trials, level of N fertilization has been shown to have significant effects on plant composition, protein digestibility, and digestibility of energy in regrowth cuttings, but little effect on intake. Increasing levels of N fertilization were associated with a decreased preference for the forage in palatability trials. In grazing trials, N fertilization generally resulted in an increased energy digestibility and intake of herbage, and in a different pattern of preference behavior. The ionic form in which nitrogen fertilizer (at equivalent N levels) was applied to grass hay was also found to affect energy digestibility, intake and palatability, although the palatability effects differed with animal species.

The effects of the addition of micro-elements in fertilizer on the nutritional quality of herbage have been examined in a number of studies with orchardgrass. Initial trials showed that treatment with Mg or with combinations of trace elements (Zn, B, Cu, Mn, Mo) gave significant increases in yield of regrowth cuttings. Differences in palatability were noted and the energy digestibility of regrowth cuttings fertilized with NPK + trace elements was significantly higher than for NPK fertilized grass.

Recent studies on micro-element (Zn, Cu, Co, Mo, S) fertilization at different soil pH's showed a consistent effect in the first year of liming on intake, although not on digestibility, of micro element treatments. Significant effects of micro elements on energy digestibility were again noted, and these differed with cutting and with soil pH. Intake of grass hay fertilized with NPK was generally high in relation to intake of hays fertilized with NPK and micro elements, and there were intake differences due to the application of individual micro nutrients.

It is suggested that effects of mineral fertilization on the nutritive quality of forages for ruminants may be reflected also in the properties of milk obtained from lactating animals consuming the forage. Preliminary trials, using a weanling rat assay technique, have shown differences in the growth rate and metabolism of young rats maintained wholly or in part on milk obtained from goats fed orchardgrass hay grown at different levels of nitrogen, and micro element, fertilization.

Application of an In Vitro Digestion Technique in Breeding and Evaluation of Forage Grasses - I. T. Carlson, W. F. Wedin, and R. L. Vetter, Iowa State University, Ames.

A technique developed by Tilley and Terry at the Grassland Research Institute in England was used to investigate variation in dry matter digestibility among

four cool season perennial grasses, smooth bromegrass, orchardgrass, reed canarygrass, and tall fescue. Variation within orchardgrass and reed canarygrass was studied also. The technique involves digestion with rumen microorganisms and acid pepsin.

Digestibility values obtained by using this procedure have agreed closely with those from conventional sheep digestibility trials and repeatability of determinations on two standard forages has been good.

As expected, the digestibility of the spring growth of the four grasses decreased with advance in maturity. The less mature growth of bromegrass and reed canarygrass was higher in digestibility than the more mature growth of orchardgrass and tall fescue on each harvest date. The digestibility of the spring growth of fifteen orchardgrass varieties was positively correlated with heading date (r = .95) and negatively correlated with stemminess (r = .84).

The digestibility of second and third cutting forage decreased with an increase in the length of the regrowth period. The rate of decline was not as great as in the first cutting. In the fall, tall fescue maintained a high digestibility, whereas the digestibility of the other grasses decreased sharply from October to December. Low digestibility of orchardgrass in the fall of 1963 in southern Iowa was associated with a high incidence of leaf disease.

A digestibility study of third-cutting forage from 20 reed canarygrass selections and their topcross progenies indicates that there are heritable differences in this trait. Correlations between percentage digestible dry matter and yield of dry matter, percentage dry matter, percentage crude protein, and palatability rating were not statistically significant.

Further correlation of results from this laboratory procedure with animal performance will be obtained in a grazing trial currently in progress.

The Cation and Organic Acid Content of Pearl Millet Forage as Related to Milk Fat Test - R. W. Hemken, N. A. Clark, B. A. Schneider, and J. H. Vandersall, University of Maryland, College Park.

Previous work from this station has established that pearl millet is responsible for lowering the butterfat content of milk produced by cows fed this forage. The earlier studies established that the basic cause of fat depression by pearl millet must be related to different factors than those responsible for the fat depression observed with high grain feeding, ground hays, etc. Buffers, sodium acetate, and supplemental alfalfa hay have all failed to return the fat test to normal levels. The pH of the rumen fluid is consistently higher with pearl millet than when feeding sudangrass and the VFA patterns do not show a large change from acetate to propionate as found with high grain feeding.

Results reported earlier have demonstrated that pearl millet differs from sudangrass in its ability to absorb minerals from the soil and accumulate nitrate nitrogen. Schneider and Clark have reported that magnesium reduces nitrate accumulation while potassium increases nitrate accumulation. A survey

of the data over several years indicated that the severity of fat depression was related to rainfall, in that the drop in fat test was greatest during dry years. Rainfall should also affect the plants ability to absorb and store certain compounds. It was hypothesized that inasmuch as the mineral content of pearl millet is much higher than that of sudangrass, the organic acids might also be influenced by fertilization level.

A green chop feeding study was conducted during the summers 1965, 1966 and 1967 in which lactating Holstein cows were fed pearl millet grown under either a high or a low level of calcium, potassium and magnesium, and compared with sudangrass under a high level of cation fertilization. During the first two years the results did not show clearly significant differences between the two pearl millet treatments. However, during 1967 the fertilization treatments of pearl millet produced a significant difference in fat test. In every year the pearl millet forage groups produced a significantly lower fat test than the sudangrass group.

Analysis of pearl millet forage for calcium, potassium and magnesium content has shown that the added fertilizer does increase the total cation content of the forage. The total acidity of the pearl millet forage samples was higher than that of sudangrass. Preliminary studies conducted on the organic acid content indicates that oxalic and succinic acids are found in consistently larger amounts than found in the control sudangrass forages. Increase in the concentration of these acids throughout the growing season were correlated with periods of butterfat depression. During these periods the concentration of the two acids was higher in the high fertility pearl millet than in the low fertility pearl millet.

Sijpesteijn and Elsden showed that succinate may be rapidly broken down by rumen microbes to form propionic acid. However, at present the level of succinate found does not seem to be high enough to contribute a sizable amount of propionate. Oxalic acid is found in relatively larger amounts than succinate and studies are planned to study the action of oxalic acid in the rumen. Rumen VFA patterns from pearl millet fed cows do show larger amounts of propionate and lower amounts of acetate than cows receiving sudangrass. The rumen VFA data from 1967 has not been completed at this time.

The results of the completed studies show that the fat depression observed when cows are fed fresh pearl millet forage is related to fertilization with cation fertilizers. Since the effect of each element (calcium, potassium, and magnesium) is confounded in the feeding studies, further work is needed to clarify the contribution of each element. In addition, the work presently planned on the infusion or feeding of organic acids should clarify their role in the fat test depression found when pearl millet is fed.

PHYSIOLOGY PANEL

Effect of Intake Level on Rumen Characteristics and Rumen Retention Time - J. W. Thomas and R. S. Emery, Michigan State University, East Lansing.

The amount of ingesta in the gastrointestinal (G.I.) tract and its characteristics have some relationship to intake level of animals. Proper quantitation

of the relationship between intake level and G.I. tract contents, particularly the rumen, might lead to an understanding of some factors regulating intake.

During the past few years data on amount of ingesta in the rumen and level of intake have been published or collected and this has been combined with data collected over several years at Michigan State University. Most data were collected using animals fed roughages only. In general there was an increase in weight of rumen contents as intake increased. The correlation coefficient was not always significant. Large variations make the simple regression equation unsuitable for predictive purposes. Multiple regressions had increased precision.

Correlations between D.M. intake and D.M. in rumen were greater and regressions more precise than those relating D.M. intake to total rumen fill. The greater the intake level the greater the D.M. concentration in rumen ingesta.

We and others have used rumen retention time defined as rumen D.M. (lb) ÷ intake D.M. (lb/day) as an indication of length of time that D.M. remains in the rumen. This has also been used for fiber and lignin. The rumen retention time for these materials was inversely related to intake (lb/cwt). Lignin remains in the rumen longer than fiber and fiber longer than D.M.

Rumen retention time of dry matter from ground pelleted alfalfa was not different from that for alfalfa hay or alfalfa silage or a mixture of alfalfa and grain. However, rumen retention time of timothy hay was usually longer than for alfalfa at comparable intake levels. Conversely, rumen retention time of "green chop" alfalfa was less than that for other feeds at comparable intake levels.

Under standardized conditions individual animal differences in the items just discussed have been noted and were of statistical significance.

Incorporation of C^{14} from Labeled Alfalfa Into Rumen Bacterial and Volatile Fatty Acid Carbon and Its Rate of Rumen Removal and Appearance in Feces - R. M. Meyer, C. L. Alexander, and E. E. Bartley, Kansas State University, Manhattan.

The efficiency of utilization of many feed nutrients is dependent on how these nutrients are metabolized and their rate of metabolism in the rumen. It has been difficult to determine the rate of rumen metabolism of a particular feed nutrient because nutrients in a given feeding lose their identity as they become mixed with nutrients already present in the rumen from previous feedings. Introduction of a small quantity of C^{14} labeled alfalfa into the rumen and measurement of the total C^{14} activity in the various fractions of rumen digesta at particular intervals permits indentification of rates of metabolism.

Carbon^{14} labeled alfalfa harvested at prebud (10-15 cm tall, very lush), bloom (late bud or early bloom, lush and 30-40 cm tall), or seed (40-50 cm tall, woody) stages of maturity was used in three in vivo trials. Incorporation of C^{14} from labeled alfalfa into rumen bacterial and volatile fatty acid carbon and its rate of rumen removal and appearance in feces was determined. Prior to feeding the alfalfa it was fractionated into acid-detergent fiber (ADF), neutral-detergent fiber (NDF), cellulose and neutral detergent solubles (NDS).

Each maturity of alfalfa was fed in separate trials to a rumen fistulated Jersey cow. Rumen samples were obtained after mixing through the rumen fistula at 1, 3, 6, 12, 18, 24, 36 and 48 hours after feeding the C^{14} labeled alfalfa. Polyethylene glycol was used to determine the total weight of rumen content and volume of rumen fluid. Carbon^{14} activity in the VFA of rumen fluid was determined. Rumen dry matter was fractionated into ADF, NDF, cellulose and NDS. Rumen bacteria were obtained by differential centrifugation of rumen fluid. Trichloroacetic acid was used to precipitate bacterial protein. Total fecal collections were made manually. Feces was fractionated into the same fractions as alfalfa and rumen dry matter.

Peak activity in rumen VFA occurred at 6 hours and peak activity in bacteria and bacterial protein occurred at 3 hours. The peak activity was followed by a logarithmic decline. There was no evidence of bursts of fermentation activity at any particular time during the decline.

Removal rates of the labeled hay fractions were logarithmic. The logarithmic nature of the removal was verified by correlation coefficients of -0.97 to -0.99.

Stage of maturity had no significant effect on rate of removal of NDS. Removal rates of dry matter, cellulose, ADF and NDF had regression slopes that indicated they were removed from alfalfa in the prebud stage at a significantly faster rate than from bloom or seed stages of maturity. No significantly different regression slopes were found between the alfalfa in bloom and seed stages of maturity.

A comparison of relative removal rates within a maturity indicates that the NDS fraction is removed significantly faster than the dry matter, cellulose, ADF or NDF fractions. The latter four fractions had removal rates that were not significantly different. The lack of more significant differences suggests a close association of all nutrients. Apparently structural constituents must be degraded to some extent before the readily fermentable cellular contents are available for microbial metabolism. There was no evidence that a fiber constituent must be conditioned for a period of time before it can be readily degraded.

The peak C^{14} activity in the NDS fraction of feces occurred 30 hours after feeding the labeled hay. The C^{14} in the fecal dry matter peaked between 30 to 36 hours. The C^{14} activity in fecal cellulose and ADF peaked between 42 to 54 hours, and in fecal NDF between 36 to 54 hours. A greater portion of the C^{14} activity in the fecal dry matter during the first 30 hours was in the NDS fraction. After 30 hours a greater portion of the activity was found in the ADF, cellulose and NDF fractions. Little activity was found in the feces before 18 hours or after 120 hours.

Metabolic Pathways of Tryptophan in the Tympanitic Bovine - Richard J. Johnson and I. A. Dyer, Washington State University, Pullman.

It has been observed that the incidence of tympanites is increased during periods of elevated temperatures. Previous studies also have suggested a slower metabolism in the tympanitic bovine (compared to normal), as well as

gross differences in tryptophan metabolism (Dyer et al. 1964. Wa. Ag. Exp. St. Bull. 43.). Investigations of the effect of temperature on urinary excretion of tryptophan and several of its metabolites seemed a logical step in elucidating the phenomena observed.

Three normal and three tympanitic steers were administered ^{14}C tryptophan (35 μC) by intramuscular injection. This was approximately 0.06 μC/Kg. body weight. The first of the two part series was conducted at an average temperature of 0°C. the second at 30°C. Urine samples were collected prior to the isotope administration and at 1, 2, 3, 4, 5, 6, 9, 12 and 18 hours after administration. Samples were frozen to retard degradation of metabolites and later thawed for analysis.

The analysis consisted of separation of tryptophan and six metabolites by paper chromatography and counting in a liquid scintillation counter. Internal standards of ^{14}C toluene were added to each sample to determine counting efficiency.

Three of the metabolites, 5-hydroxytryptophan, 5-hydroxytryptamine and tryptamine were excreted at a low level with small differences.

Tryptophan and kynurenine were excreted in greatest quantity of the metabolites assayed. At 0°C counts per minute per ml. urine for tryptophan were 872 in the tympanitic and 816 in the normal. Kynurenine excretion yielded 880 in the tympanitic and 871 in the normal. At 30°C, excretion of tryptophan was 313 CPM per ml. urine for the normal and 384 for the tympanitic while kynurenine averaged 213 CPM for the normal and 268 for the tympanitic. Indoleacetic acid and 5-hydroxyindoleacetic acid were somewhat comparable. At 0°C CPM for indoleacetic acid were 139 and 164 for the normal and tympanitic bovine, respectively. At 30°C there were 49 CPM for both. The average CPM for 5 hydroxyindoleacetic acid were 117 and 173 for the normal and tympanitic bovine, respectively, at 0°C while at 30°C the normal averaged 43 CPM and the tympanitic 49.

The data indicating differences between the normal and tympanitic in the metabolism of tryptophan support the theory of an inborn difference in metabolism of chronic tympanitics. The data suggest that the tympanitic bovine suffers greater urinary losses of compounds which may be essential for homeostasis. Increased ambient temperatures which increase the incidence of tympanites result in changes in tryptophan metabolism as well as a reduction in overall metabolic rate. The changes in tryptophan metabolism may be visualized as changes necessary to partly combat the stress of higher temperatures. The tympanitic bovine appears to be unable to adequately adjust to stresses which precipitate tympanites.

Measurement of Absorption, and Amino Acid Balance and Net Synthesis in the Bovine - D. R. Jacobson, University of Kentucky, Lexington.

The Doppler Ultrasonic Blood Flow System was employed to measure the portal blood flow rate (mean 40.9 ml/min/kg) in seven calves ranging from 3 to 22 weeks of age, which were fed milk at 5% of body weight twice daily. Absorption estimates of total sugars and glucose were made by combining portal flow and portal-carotid blood concentration differences. Telemetry of blood flow

information permitted measurements on unattended animals in a normal environment. The mean velocity of portal flow one hour postprandial was increased 9.7% by feeding. The net absorption of total sugars and glucose determined from plots of portal-carotid concentration differences over the 12-hour feeding interval combined with the portal flow rate accounted for 60% (calculated as glucose and galactose) of the lactose intake. Glucose alone accounted for 41% of the lactose intake. The difference between the total sugars and glucose absorbed indicates that some galactose or lactose per se is absorbed. The data also indicate that 20 to 40% of the fed lactose is not accounted for as absorbed sugar, which suggests that there is utilization in the gut wall. The method developed in this study, which employs the Doppler shift principle to measure blood flow, and portal-carotid concentration differences, is quite suitable for obtaining quantitative determinations of net absorption of nutrients from the gastrointestinal tract of ruminants in a normal environment.

Eight mature lactating Holstein cows were placed in metabolism units for digestibility and balance data. Twenty-four additional cows were observed for milk production and voluntary feed consumption. One-half of the above cows received a concentrate mix that contained 0.10% sulfur and the other half the same concentrate supplemented with sodium sulphate to contain 0.18% sulfur. Both groups were fed the same corn silage (0.09% sulfur, dry basis), and the respective concentrate mix ad lib. at a ratio of 6.3 to 1.0. The balance data were obtained at 80% of ad lib. intake. The cows were in negative sulfur balance, but positive nitrogen balance. The supplemental sulfur was inefficiently utilized as indicated by the increased urinary excretion of sulfur and by no effect on sulfur balance. Balance of amino acids was very similar for both groups. All animals were essentially in balance for methionine, cystine, and tyrosine, negative balance for glutamic acid and lysine, and in positive balance for histidine, arginine, aspartic acid, threonine, serine, glycine, alanine, valine, isoleucine, leucine, phenylalanine, and proline. The data suggest that inadequate rumen net synthesis of certain amino acids limits voluntary feed intake and milk production of high producing cows in which 45% of the nitrogen of the diet is non-protein nitrogen.

MICROBIOLOGY PANEL

Dietary Influences on B-Vitamin Concentrations in Ruminal Fluid - B. W. Hayes, G. E. Mitchell, Jr., C. O. Little, and N. W. Bradley, University of Kentucky, Lexington.

Microbiological assays for thiamine, riboflavin, biotin, pantothenic acid, folic acid, niacin and vitamin B_{12} were conducted on samples of ruminal fluid taken from yearling steers by stomach tube. Eight steers, fed in pairs, were assigned to each of the following treatments for 56 days: flaked corn, ground corn, flaked corn and long hay, ground corn and long hay, flaked corn and ground hay, and ground corn and ground hay. Corn was self-fed, 1.8 kg. alfalfa hay was fed per head per day, and supplements containing soybean meal, vitamins A and D and minerals were fed at levels calculated to meet recommended requirements and equalize intake of supplemented nutrients. Biotin was the only vitamin studied which was not significantly ($P>.05$) affected by

treatment. Concentrations of thiamine, pantothenic acid, folic acid, niacin and vitamin B_{12} were greatest when all-concentrate diets were fed. Riboflavin concentrations were greatest when ground hay was fed.

In vitro experiments were conducted to compare B-vitamin synthesis during starch digestion with synthesis during cellulose digestion at initial pH values of 6.8 and 5.5 and to study possible effects of adaptation to hay, mixed and all-concentrate diets on B-vitamin synthesis. Concentrations of thiamine, folic acid and pantothenic acid were not significantly affected by change from starch to cellulose or by initial pH of the inoculum. Concentrations of riboflavin, niacin and vitamin B_{12} were significantly ($P<.05$) greater after 9-hr. incubations with starch than after 24-hr. incubations with cellulose. Niacin concentrations were significantly higher when initial pH was 6.8. In vitro B-vitamin synthesis was lowest when microorganisms were obtained from a hay-fed steer, highest when microorganisms were obtained from a steer fed all concentrates, and intermediate for the mixed diet. Most B-vitamin concentrations were highest when in vitro substrates corresponded to the rations of the steers from which ruminal microorganisms were obtained.

Lipids of Selected Rumen Ciliate Protozoa - Barbara J. Corpening, R. Berube, A. R. Abou Akkada, L. R. Fina, and W. E. Klopfenstein, Kansas State University, Manhattan.

Lipids were ether extracted from pure washed suspensions of Epidenium, Entodenium, holotrichs and mixed protozoa. They were methylated and analyzed chromatographically. Palmitic acid constitutes the major part of the Epidenium and the holotrich lipids, whereas stearic acid is the principle component of the Entodenium. Unsaturated fatty acids were found in significant amounts in these protozoa. Oleic acid was the principle unsaturated fatty acid in Entodenium, Epidenium, and mixed protozoa. Linolenic acid was found only in Epidenium. The endogenous lipid of protozoa contained a significant quantity of volatile fatty acids. Acetic acid was the main acid in the holotrichs and mixed protozoa, and butyric was high in Epidenium. It was concluded that the protozoa, especially Epidenium, play an important role in the lipid metabolism of the host ruminant.

Reductive Carboxylation Reactions in Ruminal Amino Acid Biosynthesis - M. J. Allison, I. M. Robinson, and J. L. Peel, National Animal Disease Laboratory, USDA, Ames, Iowa and Food Research Institute, Norwich, England.

The branched-chain amino acids and phenylalanine and tryptophan are deaminated and decarboxylated to produce isovalerate, isobutyrate, 2-methylbutyrate, phenylacetate and indoleacetate. We have shown that all of these acids are carboxylated by the mixed microbial population of the rumen (incubated in vitro) to resynthesize the carbon skeleton of the original amino acid.

A survey was made of representative strains of the more important species of ruminal bacteria to determine which organisms utilized this carboxylation mechanism for amino acid biosynthesis. The organisms used were isolated and

described by Marvin P. Bryant. They were grown in media containing carbon-14 labeled acids and radioactivity in individual amino acids from the bacterial protein was determined.

A wide variety of morphologic (rods, spirochetes and cocci) and physiologic (cellulolytic, amylolytic, proteolytic and methanogenic) types of bacteria used one or more of the acids to synthesize the corresponding amino acid. None of the organisms examined used all 5 of the acids. Phenylacetate was used for phenylalanine biosynthesis by eight of the eleven species studied. Isoleucine was synthesized using 2-methylbutyrate by seven of fourteen species. Included in the group using 2-methylbutyrate were two species (Bacteroides succinogenes and a Borrelia sp.) that did not use the other branched-chain acids to synthesize amino acids. This suggests a specificity of the carboxylation enzyme systems. Ruminococcus albus was the only species studied that utilized indoleacetate in biosynthesis of tryptophan. The results suggest that these reactions are quantitatively significant in the rumen and we have obtained evidence that these reactions are also functional in several other anaerobic environments. The possibility that amino acids other than the five mentioned here are synthesized by similar reactions is being investigated.

The biochemical mechanism of the carboxylation reaction has been studied using cell-free extracts of Bacteroides ruminicola and Peptostreptococcus elsdenii. Synthesis of the valine carbon skeleton, using isobutyrate, was dependent upon ATP, CoA and pyruvate in extracts from B. ruminicola. P. elsdenii extracts contain a hydrogenase and hydrogen replaced the requirement for pyruvate. With extracts from P. elsdenii treated with DEAE cellulose, isobutyrate carboxylation was dependent upon the addition of ferredoxin or flavodoxin. Ferredoxin, however, could not be detected in cells of B. ruminicola so perhaps some other electron carrier is involved in that organism. The available evidence suggests that the carboxylation reactions are similar to the pyruvate synthase system that has been found in certain clostridia and in photosynthetic anaerobes.

Studies on the Mechanism of Pentosan Degradation by Cellulolytic Rumen Bacteria - B. A. Dehority, Ohio Agricultural Research and Development Center, Wooster.

Although certain strains of cellulolytic rumen bacteria cannot utilize isolated hemicelluloses or xylan as a source of energy, all strains examined can degrade or solubilize these materials from an 80% ethyl alcohol insoluble to a soluble form. Centrifugation and washing of the cellobiose grown bacterial cells did not affect the rate or extent of utilization or degradation or both. When the level of a nonutilizing culture inoculum (either normal or washed) was doubled, a corresponding increase in the initial rate of degradation was observed. Using a nitrogen-free medium, utilization of xylan was almost completely inhibited for a utilizing strain while degradation by either type of organism was not markedly affected. Cellobiose medium cell-free culture filtrates from a nonutilizing strain were able to degrade or solubilize xylan. The percent degradation increased with volume of cell-free filtrate, and all activity was lost when the filtrate was boiled. No utilization (loss in total pentose) was observed with cell-free filtrates from utilizing or nonutilizing

strains. The release of free hexose from insoluble cellulose by culture filtrates from a nonutilizing strain was very limited. On the other hand, carboxymethylcellulose (CMC-70L) and cellulodextrins were solubilized in 80% ethyl alcohol by filtrates from both types of organisms. Similar enzyme activity was obtained in cell-free culture filtrates from four additional strains of cellulolytic rumen bacteria (one xylan utilizer and three nonutilizers). When the assays were carried out aerobically, CMC-70L solubilization was reduced to a much greater extent than xylan or cellulodextrin solubilization. The enzyme or enzymes responsible for the degradation of hemicellulose by cellulolytic rumen bacteria unable to utilize the hemicellulose as an energy source appear to be constitutive in nature and this activity may be a nonspecific action of a β-1,4 glucosidase or cellulase.