REPORT ON

CONFERENCE ON RUMEN FUNCTION

held at
La Salle Hotel, Chicago, Illinois
December 3-4, 1975

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

General Chairman	-- C. R. Richards --------	CSRS, USDA
(a) Agronomic		J. C. Burns (NC)
(b) Microbiology		M. P. Bryant (IL)
(c) Nutrition		J. T. Huber (MI)
(d) Physiopathology		W. M. Wass (IA)

AGRONOMIC

Further on an Alkali-Swelling Test with Cotton Fiber for the Experimental Study of Cellulose Decomposition in Rumen Fluid - Paul B. Marsh, Marion E. Simpson, and George V. Merola, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

This report supplements information presented at the 1973 Conference. An alkali-absorption ("alkali-centrifuge" or "AC") test has proven to be a practical index of cellulose digestion in cotton fiber incubated in rumen fluid at levels of digestion too low for measurement by direct weight loss. The AC results tend to move into an unresponsive "ceiling" range when direct weight losses exceed 5 - 15%.

The minimum incubation time required for major increases in AC value has been lowered to 6 hours by increase in the rumen fluid-to-sample ratio and more complete anaerobiosis. It has been lowered further to 2 hours by the use of rumen fluid pre-incubated with cotton fiber to increase its potency before the test incubation.

In experiments with graded concentrations of additives, parallel changes in cellulose digestion have been observed in comparative results by direct weight loss and by increase in AC value; additives included antibiotics, surfactants, and acetic acid. The AC test may thus be used as a check on results by direct weight loss with short-and-long incubation periods or as a sole measure when levels of digestion are all very low.

The early increases in AC values are accompanied by high absorption of the dye Congo Red after alkali-swelling, followed in sequence in longer incubation by surface pitting, localized holes, and finally generalized disintegration of the fiber.

The alkali-centrifuge test is simple, rapid, and inexpensive, and requires no specialized equipment. A fairly considerable background is to be found in the literature on its methodology and its use to measure effects of fungi and fungal cellulases on cotton fiber.

Infrared - New Approach to Forage Quality Evaluation - John S. Shenk, Department of Agronomy, Pennsylvania State University, University Park, Pennsylvania.

One of the important objectives in forage research has been the development of rapid, accurate, and inexpensive laboratory procedures to determine the quality or feeding value of forage. Currently laboratory procedures in use do a satisfactory job of describing forage quality; however, if progress is going to continue in this sector of agriculture, new and more rapid techniques will be needed.

The first breakthrough in new instrumentation was demonstrated April 28, 1975 at a meeting of the Hay Marketing Committee sponsored by the American Forage and Grassland Congress by its inventor, Karl H. Norris. Norris is chief engineer of the Instrumentation Laboratory, Beltsville. These initial studies of the near infrared spectra (1.4 to 2.4 μm) were conducted with 87 samples of dry ground forage. Temperate forage species analyzed were alfalfa, tall fescue, smooth bromegrass and alfalfa bromegrass mixtures. These forages had been preserved as hay, silage, and fresh frozen forage. Tropical species included bermudagrass and Pangola digitgrass. Correlations for laboratory measurements of chemical composition were 0.99 for crude protein, 0.98 for neutral detergent fiber, 0.96 for acid detergent fiber, 0.96 for lignin, and 0.95 for in vitro rumen digestion. Animal response correlations were 0.88 for dry matter digestibility, 0.80 for dry matter intake, and 0.85 for digestible energy intake. Since one of the key components of the infrared analyses is a computer, the data from the instrument can be used directly for ration formulation, forage management or plant breeding research.

We were so impressed with the potential of this device that Penn State has purchased this instrument with a Specific Cooperative Agreement from ARS-USDA. The instrument is currently housed at the USDA Regional Pasture Research Laboratory. Our first efforts will be to verify the initial discoveries followed by assessing the potential of the instrument to determine other chemical constituents and animal response factors. If these verification studies are successful we will place the instrument into routine service on a research basis as part of our Cooperative Crop Quality Laboratory between the Pasture Laboratory and the College of Agriculture. Research will then be initiated into the basic aspects of the procedure as well as studies into new applications of the technology.

Breeding for Improved Forage Quality in Reed Canarygrass - A. W. Hovin, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota.

Reed canarygrass is a widely adapted and high yielding forage species but is also known to be unpalatable to livestock. The discovery by Simons and Marten (1971) that palatability in this species was adversely associated with total alkaloids gave impetus to the breeding program I will describe. Our cooperative effort to improve the quality of reed canarygrass forage has concentrated on alkaloids, cell wall constituents (CWC) and in vitro digestibility (IVDDM).

We have demonstrated that heritability of alkaloid concentration is moderately high and is influenced by environment particularly at the lower range of concentration (Barker and Hovin, 1974). In our breeding program we currently use parent clones with alkaloid concentration below 0.10% of dry wt determined on regrowth forage. We may need to select for a lower concentration in order to exploit the improvements in palatability and intake. Our research so far has not uncovered any adverse relationship between alkaloid and other quality factors of reed canarygrass forage. We have observed animal performance differences due to alkaloid type (Marten, Jordan, and Hovin, 1975). Gramine has caused less diarrhea to grazing sheep than tryptamines and β-carbolines. We are studying the mode of inhertiance of these indole alkaloids.

Because voluntary intake is affected by CWC, we have become interested in breeding for lower CWC concentration. Heritability estimates suggested that CWC was predominantely under additive genetic control. Correlations between CWC and IVDDM were in the -0.68 to -0.77 range. Multiple correlation of cell solubles (100-CWC) and IVDDM with forage yield (range 0.47 to 0.50) suggested that progress can be made in selecting for a lower CWC without adversely affecting forage yield and digestibility (Hovin, Marten, and Stucker, 1975).

Our heritability estimates for IVDDM obtained for regrowth forage show a high proportion of non-additive genetic variance of the total genetic variance (Hovin, Stucker, and Marten, 1974). We have observed an expected negative relationship between IVDDM and degree of maturation.

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Nutrient Composition of Crop Residues as Related to Utilization and Beef Cattle Requirements - R. L. Vetter, Department of Animal Science, Iowa State University, Ames, Iowa.

The nutrient composition and quality enhancement of crop residues (corn, soybean and sorghum) were discussed. With harvested residue feeds, protein and mineral contents are most variable, depending on grain maturity, plant variety and nutrition, harvesting methods and environmental factors. Crude protein content of residues harvested at maturity is not adequate to supply maintenance requirements of mature ruminants (except sorghum stover under certain condition About 20% of the nitrogen in stover is bound with lignin and is unavailable. About 50% of the nitrogen availability is dependent on the solubilization of t

hemicellulose/cellulose fraction. The nutritional enhancement of residue feeds is dependent on the associative effects of nitrogen and energy availability. These limits are narrower for residues, and therefore, much more critical than for higher quality feeds. Phosphorous levels are deficient in residue feeds, averaging 0.1% of dry matter. The percentage of leaf material in the harvested feed is the main determinant of mineral content.

Relative to higher grain costs, chemical (alkali) treatment and processing shows increased potential. A total assessment of labor, energy expenditures and handling hazards may not lend it to routine on-farm application. More emphasis should be given to genetic selection for lower lignin content or more digestible cell wall structure. A large potential exists for enhancement of residue feeds through fermentation processes.

Ozone, and Sulfur Dioxide - Their Effect on Nutrient Composition of Forages - Robert K. Howell, Plant Stress Laboratory, Plant Physiology Institute, Beltsville, Maryland.

The objective is to answer the question, "What is the impact of air pollution on the nutritional value of forage?" Eight alfalfa entries, four resistant and four susceptible to anthracnose were treated with ozone (03) 20 pphm for 4 hours or with 1.5 ppm sulfur dioxide (SO_2) for 2 hours. Tissues were harvested 36 hours later and were analyzed for International Units (IU) of Vitamin A, percent protein, fat, carbohydrate, and crude fiber. The results were compared with results from similar tissues not treated. All analyses were determined by AOAC methods. Results demonstrate that degree of tolerance to anthracnose had no significant influence on concentrations of constituents assayed. Also, there was no significant interaction of alfalfa entries by parameter. Vitamin A was equally and significantly reduced by SO_2 and O_3; Control 23,148, SO_2 13,213, and O_3 13,332 IU/100 gms. respectively. Percent protein was significantly less in O_3 treated tissues 18.5% than in SO_2 treated tissues 21.6%, but both were significantly lower than in nontreated alfalfa 28.7%. Percent fat was significantly reduced by both SO_2 1.1% and O_3 1.1%. Nontreated tissues contained 1.5% fat. Percent total carbohydrate was significantly greater in SO_2 73.9% and O_3 75.1% treated than in nontreated 66.2%. Percent crude fiber was significantly greater in SO_2 12.5% and O_3 14.4% treated than in nontreated tissues 10.4%.

One variety of red clover, Kenland, was treated with either O_3, 0.5, 1.0, or 1.5 ppm SO_2 for 4 hours and tissues were analyzed for the same components as described for alfalfa. The lowest SO_2 level effected little change in concentrations of the nutritional components. Vitamin A was significantly reduced by 1.0 or 1.5 ppm SO_2, percent fat was significantly increased, and percent protein was reduced by 1 ppm, but was increased in tissues treated with 1.5 ppm SO_2. Percent crude firber too increased significantly in tissues treated with 1 ppm but was less than in control or in tissues treated with 1.5 ppm SO_2.

In another experiment Kenland red clover was cultured in either carbon filtered or non-filtered greenhouses. Tissues were harvested after 30 days treatment during July 1974 from both environments and analyzed for the same components. Vitamin A and percent protein were significantly reduced by elements in non-filtered air 23,779 vs 17,426 I.U. and 25.6% vs 17.7% respectively. Percent fat,

carbohydrate, and crude fiber were significantly increased in tissues grown in non-filtered air.

Conclusions - The effect of air pollutants on nutritional components in forage crops has received little attention. This data clearly indicates that important nutritional constituents are significantly influenced by ozone, and sulfur dioxide. Both pollutants appear to induce the same changes with respect to parameters assayed. The data also indicates that at least in red clover, trends in changes of nutritional components are the same in tissues exposed to short term fumigations in growth chambers or to long-term intermittant exposures in a greenhouse containing non-filtered air. Research on air pollutant induced nutritional changes needs to be expanded to determine what the actual effects of such exposures and resultant modification may be on actual feed values or total digestible nutrients of forages. Answers to such questions as do air pollutants increase lignin content of forages, change concentrations of saponins, and modify forage susceptibility to micro-organisms in both the field and in the rumen, would be useful.

MICROBIOLOGY

Ultrastructure of the Association with and Subsequent Degradation of Forage Tissue Types by Rumen Microorganisms - Danny E. Akin, Henry E. Amos, Franklin E. Barton, II, and Donald Burdick, Field Crops Marketing and Utilization Research Laboratory, Richards B. Russell Agricultural Research Center, ARS, USDA, Athens, Georgia.

Rumen bacteria associated differently with various tissue types in forage grass leaves during degradation as shown by electron microscopy. Cell walls of the easily digested mesophyll and phloem appeared to be degraded at times without attachment of bacteria as shown by non-uniform eroded zones away from the bacteria. The more slowly digested bundle sheath and epidermal walls appeared to be degraded after attachment, in many cases, of rumen bacteria to the forage cell walls as shown by uniform eroded areas in the shape of (and surrounding) the attached bacteria. Usually, lignified vascular and sclerenchymal tissue (except for the periphery of sclerenchyma) were not degraded by normal rumen microflora, and attachment to those tissues was not prevalent.

The importance of the various tissue types to digestion is indicated by the fact that forage species differ in amount of slowly and rapidly degraded tissues. Perennial warm-season grasses, considered to be less digestible than cool-season forages, possessed more of the slowly digested tissues (48% for 4 warm-season grasses vs. 26% for 6 cool-season grasses), to which bacteria attached prior to degradation. The fact that the percentages of nondegradable, lignified tissues did not differ substantially (12% for warm-season vs. 14% for cool-season grasses) suggested that the association of rumen bacteria to degradable tissues of various digestibilities was important to the rate of forage digestibility.

Tissues common to all grass leaves appeared to have inherent differences in cell walls among forages that affected digestibility as shown by a differential rate of degradation of parenchymal bundle sheaths. In addition, a differential response of tissues in bermudagrass and fescue to extration with acid detergent reagent (cetyltrimethylammonium bromide) indicated inherent differences in cell walls.

Bacteria of different morphologies differed in the manner of attachment to plant walls as shown by ruthenium red-stained samples of degraded leaves. Cocci attached to plant walls during degradation by a distinct, capsule-like material. Conversely, bacilli were found to attach to plant walls without visible amounts or by small amounts of extracellular material. Bacilli possessing an irregularly folded, electron dense outer layer (or layers) about 15 nm thick but without visible fibrous, extracellular material adhered closely to plant cells during degradation such that irregular shapes of the bacteria resulted.

In recent examinations of in vitro and in vivo digested leaves, a small (less than 1 um) filamentous, branching microbe was found that degraded the slowly digested and lignified sclerenchymal tissues. This microbe possessed structural features similar to actinomycetes. The degradation of sclerenchyma represents an important biochemical function in the rumen which may affect the extent of digestion.

A Basal Medium for the Selective Enumeration of Rumen Bacteria Utilizing Specific Energy Sources - B. A. Dehority and Jean A. Grubb, Department of Animal Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

A 40% rumen fluid basal medium has been developed, which without added substrate will support growth of about 10% or less of the total colony count obtained with 40% rumen fluid-glucose-cellobiose-starch-agar medium (RGCSA). The basal medium is prepared by anaerobic incubation of all ingredients in RGCSA medium, except the carbohdrates, Na_2CO_3 and cysteine, for 7 days at 39°C. After incubation, substrates, as required, Na_2CO_3 and cysteine are added and the medium is tubed and sterilized as in normal medium preparation. When xylose was included with glucose, cellobiose and starch as added carbohydrates in the incubated medium, total colony counts were comparable to those obtained with RGCSA medium. The addition of specific carbohydrates or other substrates as energy sources to the basal medium suggested that the percentage of the bacterial population capable of utilizing these energy sources was influenced by ration of the animal; however, considerable animal variation and day to day variation in a given animal was observed. Comparison of the population in animals fed either orchardgrass hay (OG) or 60% corn - 40% orchardgrass (60-40), indicated little or no difference for the percentage of bacteria utilizing glucose, pectin, xylan or mannitol. Increases in the percentage of xylose, cellobiose, glycerol and lactate utilizing bacteria occurred with the OG ration, while the percentage of starch digesting bacteria was increased significantly ($P<.01$) in the 60-40 fed animal. A limited number of bacterial strains were isolated from the basal medium without added substrate, most of which were atypical with respect to the predominant rumen bacteria. Growth of these strains, even in complex media, was very limited, slow and in some cases, with 10% rumen fluid in the medium, unaffected by the absence of added carbohydrate.

Metabolism of Some Essential Amino Acids by Rumen Microbes - T. Sutardi and L. D. Satter, Dairy Science Department, University of Wisconsin, Madison, Wisconsin

A series of continuous culture incubations of L- (U-^{14}C) amino acids (AA) in rumen fluid was conducted to determine the distribution of amino acid carbon into

volatile fatty acids (VFA), Microbial Protein, CO_2, and a residual soluble fraction. Sixteen grams of dry matter containing 85% alfalfa leaf meal, 10% ground corn, and 5% dry molasses was added daily to the fermentor. Bicarbonate buffer, pH = 8.2, was continuously infused to give a volume turnover rate of 1.4/day. Fermentor volume was 500 ml. Fermentation period were 10 days, with the first 4 days being an equilibrium period.

Distribution of label following continuous infusion of 1.42 $\pm$ 0.10 μC of L-(U-^{14}C)- LEU was: 31.6 $\pm$ 2.76% in VFA; 26.0 $\pm$ 1.06% in Microbial Protein; 10.3 $\pm$ 0.66% in CO_2; 3.40 $\pm$ 0.22% in CH_4; and 22.4 $\pm$ 4.37% in the residual soluble fraction.

Continuous infusion of 2.5 μC and single injection of 12.5 uC of L-(U-^{14}C)-labeled LEU, ILE, VAL, THR, MET, or LYS, revealed differences ($P<0.05$) among AA in the catabolism of carbon chains. Incorporation of label from branched-chain AA into Microbial Protein was higher (30.7%) than that of the other three AA (22.6%). About 59.7% of infused activity of LYS was recovered in the VFA, almost twice as much as that of the other AA. Methionine gave a consistently higher activity in the CO_2 fraction compared to the other AA (15.9% vs. 7.60%).

Seperation of individual VFA revealed that the majority (48.4%) of label recovered in the total VFA fraction from branched-chain AA and THR was recovered in the VFA having one carbon less than the corresponding AA. With MET, 45.5% and 45.0% of VFA activity was recovered in the C_2 and C_3 fraction. With LYS, about 65.7% of VFA activity was recovered in the C_4 fraction.

An attempt was made to quantitate the amount of free α-ketoacids in the fermentor effluent. The α-ketoacids were seperated by paper chromatography as the hydrazone derivative of 2,4-dinitro-phenylhydrazine, using 70% n-Putanol, 10% Ethanol, and 20% of 0.5 N NH_4OH as a solvent system. Due to the similarity in R_f values, and due to ketoacid isomer formation, the resolution between the α-keto analogues of ALA, THR, VAL, ILE, and LEU were not good. Despite the poor resolution, elution of the tentatively identified ketoacid revealed that about 5- 13% of the activity infused as the precursor amino acid was recovered as the α-keto acid derivative.

Effects of Urea-Nitrogen to Amino Acid-Nitrogen Ratios Upon Rumen Microbial Growth Yields in Vitro - W. J. Maeng, R. L. Baldwin, and J. G. Morris, Department of Animal Science, University of California, Davis, California.

Effects of different ratios of urea-nitrogen to amino acid-nitrogen for rumen microbial growth and incorporation of a UL-^{14}C-amino acid mixture into microbial cells were studied with washed cell suspensions in vitro. Cellular rate of glucose consumption and incorporation of UL-^{14}C-glucose into microbial cells, carbon dioxide and organic acids were also studied. Fermentation flasks contained 100 ml of culture media consisting of 100 mmoles of glucose, starch or cellobiose, 54 mg of nitrogen as urea, amino acids or combination of both in varying proportions and 15 ml of washed cell suspensions. Final volumes were adjusted to 100 ml with rumen buffer solution. The optimum ratio of urea-nitrogen to amino acid-nitrogen for rumen microbial growth was 75:25 when glucose, soluble starch and cellobiose were the energy sources. With this

amount of amino acids, an average of 53% of added amino acids was incorporated into microbial cells, 14% was fermented to carbon dioxide plus volatile fatty acids, and 33% remained in the supernatant. Both 100% urea-nitrogen and 100% amino acid-nitrogen in growth media were unfavorable for maximal microbial growth. Specific growth rates of rumen microbes were .104 and .203 and mean doubling times were 6.7 hours and 3.4 hours, respectively in 100% urea-nitrogen and 75% urea-nitroben plus 25% amino acid-nitrogen in growth media with glucose as energy source. Microbial dry weight in mg per 100 mg glucose used, and per mmole of adenosine triphosphate generated were 19.3 and 15.4 with 100% urea-nitrogen, and 24.4 and 20.6 with 75% urea-nitrogen plus 25% amino acid-nitrogen. Cellular rates of glucose consumption and percentages of catabolized glucose incorporated into microbial cells, carbon dioxide and organic acids did not differ between treatments and averaged .015 mmole per mg cells per hour and 19.9%, 7.8% and 64.4%, respectively.

Oxalate Degradation in the Rumen - M. J. Allison, I. M. Robinson, E. T. Littledike, and H. M. Cook, National Animal Disease Center, USDA, ARS, Ames, Iowa.

The rate of oxalate degradation by ruminal contents from cattle and sheep increased significantly 2 days after Halogeton glomeratus (12% oxalate) was added to the diet. When ruminal contents was incubated in a continuous fermenter increased rates of oxalate degradation were observed when gradually increasing quantities of halogeton were fed to the fermenter and also in response to infusion of sodium oxalate. The fermenter was fed alfalfa daily during experiments when sodium oxalate was infused. Rates of oxalate degradation by unadapted populations were less than 0.2 umoles/ml/hr. In response to oxalate infusion, these rates increased to 6 umoles/ml/hr or higher.

When a linearly increasing gradient of Na-oxalate was infused, oxalate began to accumulate in the fermenter but the oxalate concentration was reduced to a very low level as the rate of oxalate degradation increased.

In the oxalate-adapted fermenter with a turnover time of 16 hours, the ciliate protozoa were absent but a diverse bacterial population which degraded oxalate was maintained for as long as four weeks. Volatile acid proportions in the fermenter were similar before and after adaptation to oxalate. The quantity of CH_4 produced by the fermenter was greater after adaptation to oxalate.

Contents from the oxalate-adapted fermenter produced additional CH_4 in proportion to the quantity of oxalate added. Oxalate metabolism was reduced when formate or inhibitors of methanogenesis (benzyl viologen or chloroform) were added.

Cell-free extracts from cells in the adapted fermenter produced CO_2 and formate from oxalate. Activity was dependent upon acetyl CoA or an acyl CoA generating system. Enrichment cultures that degrade oxalate have been obtained but attempts to isolate the bacteria responsible for oxalate degradation have not been successful.

Rumen Bacteria of the Normal Rumen and of the Rumen of Sheep Fed Slow-Release Non-Protein Nitrogen - Coleman, R. N. and L. P. Milligan, Department of Animal Science, College of Agriculture, University of Alberta, Edmonton, Alberta; J. W. Costerton, Department of Biology, University of Calgary, Alberta; and K. J. Cheng, Research Station, Canada Agriculture, Lethbridge, Alberta, Canada.

We have examined the cell surfaces of rumen bacteria in pure cultures, in the normal rumen of cows and sheep, and in the rumens of sheep maintained on a mixed diet of hay and slow-release non-protein nitrogen (glucosyl-urea).

Of the six species examined in pure culture, all showed the presence of extracellular coats or slime structures. One species, Bacteroides ruminicola, had a coat composed of close-packed globular units, reminiscent of the protein coats of aquatic bacteria while all others had fibrous slime layers whose reaction with ruthenium red stains indicated that they were composed of anionic carbohydrates. The fibrous carbohydrate capsules were sometimes very thin (e. g. Bacteroides succinogenes) and sometimes very substantial (e. g. Ruminococcus albus) and we have noted that their formation is enhanced by high energy levels (a high C/N ratio) and that they can function in mediating bacterial adhesion to solid surfaces.

An examination of the rumen bacteria of cows fed on hay and on a coarse concentrate diet showed that most of the cells were gram-negative, and that all cells had a coat or a fibrous capsule. Several morphologically distinct types of capsules were noted among which were concentric and radial arrangements of fibers. In several instances, capsules were seen to mediate bacterial attachment to fragments of plant cell wall. The rumen bacteria of sheep fed on hay resembled those of cows fed on hay in that most were gram-negative, many similar capsule morphologies were noted, and slime was produced by all cells but confluent slime was not formed.

We examined the rumen bacteria of glucosyl-urea (G. U.) fed sheep to determine whether this dietary shift selected for a different bacterial population or modified slime production by the component cells. We found that the bacteria of G. U.-fed sheep were predominantly gram negative, including many morphological types seen in the normal rumen, and produced discrete but not confluent masses of extracellular slime. The only unique feature of this bacterial population was the storage of above normal amounts of reserve carbohydrates within the cytoplasma of the component cells.

We conclude that the surface of bacteria in the normal rumen is protected by a layer of fibrous carbohydrate slime or, less commonly, by a protein coat, and that this situation also obtains in the rumen of G. U.-fed sheep.

The Role of Rumen Bacteria and Saliva in the Development of Viscosity in the Bovine Rumen - Cheng, K. J., R. Hironaka, C. B. Bailey, Research Station, Canada Agriculture, Lethbridge, Alberta, Canada, and J. W. Costerton, Department of Biology, University of Calgary, Calgary, Alberta, Canada.

When a high energy concentrate feed is fed in a fine particle size (300 μm) and in a coarse particle size (715 μm) cows fed the coarse feed had normal rumen fluids while cows fed the fine feed showed high soluble carbohdrate and high viscosity in the rumen and their rumen fluid was foamy and very viscous. Electron

microscopy showed that bacterial cells in the rumens of cows fed the coarse feed were often surrounded by discrete capsules of fibrous carbohydrate but that bacteria from the rumens of cows fed the fine feed were enmeshed in a continuous mass of fibrous slime which often enclosed microcolonies of morphologically similar cells. This confluent slime mass was clearly of bacterial origin and appeared to result from an "overproduction" of fibrous capsular material.

Because we theorized that rumen bacteria may overproduce extracellular carbohydrate slime when supplied with high levels of available carbohdrate we tested Streptococcus bovis with three levels of sucrose (0.5%, 3.0% and 6.0%) and found that the lower sucrose levels promoted growth and normal viscosity while the higher level (6%) produced a spectacular increase in viscosity. This viscosity was reversible by dextranase digestion and the material responsible thus appears to be a dextran. The capsular material of Streptococcus is distinct from the net-like mass of dextran fibers which occupies much of the intercellular space in the very viscous 6% sucrose cultures.

While we conclude that bacterial slime formation is the major factor in the development of high viscosity in the bovine rumen we also used periplasmic alkaline phosphatase (APase) as a marker to detect bacterial cell breakdown which would contribute nucleic acids and other polymers to the viscous rumen contents. Electron microscopy showed a degree of bacterial breakdown and a release of APase, and assays of the enzyme detected higher overall levels both in the rumen bacteria of cows fed the fine feed and in the cell-free rumen fluid of these cows. We suggest that elevated APase levels, and especially elevated cell-free APase levels could be used to indicate the onset of elevated rumen viscosity and of conditions associated with feed lot bloat.

We examined the role of bovine saliva in the development of elevated viscosity by obtaining samples of saliva and incubating them under different gaseous phases and with rumen fluid. We conclude that the viscosity of bovine saliva is reduced to that of the rumen fluid itself by a cell-free enzyme in the rumen fluid.

In conclusion we attribute elevated rumen viscosity largely to bacterial slime production and partially to bacterial lysis, in the system we have studied, and we discount bovine saliva as a factor in rumen viscosity.

NUTRITION

The Effect of Rumensin on Performance of Beef Cattle - E. L. Potter, A. P. Raun, L. F. Richardson, H. Brown and C. O. Cooley, Lilly Research Laboratories, Greenfield, Indiana.

An in vitro ruminal test system was used to determine the effects of different compounds upon the end product volatile fatty acids. Monensin (1 ppm) decreased acetic acid, increased propionic acid, decreased butyric acid without affecting total acid production. These same shifts were observed in fistulated cattle when fed monensin. Nineteen feedlot experiments have been conducted and summarized to define the effects of RUMENSIN on gain, feed intake and feed efficiency of cattle. At 33 ppm RUMENSIN improved feed conversion 10.5 percent

without affecting gain. Feed consumption of cattle fed RUMENSIN with high grain rations was decreased 10 percent. The reduction in feed intake produced by RUMENSIN was observed to diminish during the feeding period. Carcass evaluations indicated that RUMENSIN caused no change in carcass composition or quality factors. Twelve pasture and greenchop experiments have been completed and summarized. RUMENSIN was fed via a smal quantity of supplemental feed each day. In contrast to the feedlot results, RUMENSIN at 200 mg/head/day increased daily gain of the pasture cattle by 20 percent while total dry matter intake was similar to that of the controls. Feed efficiency was improved in both feedlot and pasture fed cattle. The gain response attributed to RUMENSIN in pasture cattle appears to be the result of the animal obtaining more net energy from its ration. In comparison the feedlot animal obtains the same total quantity of net energy on less feed.

The Effect of Rumensin on Ruminal Parameters of Beef Cattle and Sheep - L. F. Richardson, A. P. Raun and E. L. Potter, Lilly Research Laboratories, Greenfield, Indiana.

RUMENSIN decreased the molar percentages of acetic acid and butyric acid and increased propionic acid in 19 feedlot trials and 12 pasture trails. Total acid production was not consistently changed by RUMENSIN. These changes in proportions of volatile fatty acids have been investigated in relation to the effect of monensin on ruminal protozoa and in respect to the biochemical pathway via which propionate is produced. Results indicate that protozoal numbers may be decreased by monensin; however, this appears to be dependent upon diet. Increases in the relative proportions of propionic acid were observed when monensin was fed to defaunated lambs. Monensin in vitro resulted in a larger proportion of propionic acid to be formed via the non-randomizing pathway. Other ruminal observations indicate that monensin increased ruminal pH slightly and decreased ruminal ammonia.

Metabolic measurements which appear to have changes as a result of these ruminal effects are: increased plasma glucose and increased plasma insulin; most probably the result of an increased contribution to glucose.

Tallow and Urea Interactions In Vivo and In Vitro - D. C. Church, Department of Animal Science, Oregon State University, Corvallis, Oregon.

Feedlot data from several different experiment stations indicate, in some circumstances, that negative interactions may occur when cattle are fed urea and tallow. These reactions have been demonstrated with animals fed high corn or high barley rations. Data on digestibility of rations reported in this paper suggest that rations with 1.6% urea and 5% tallow may allow tallow-urea interactions which result in a reduction of digestibility of dry matter, N and energy and a reduction in N retention as compared to similar rations containing urea or tallow. Feeding protected tallow resulted in normal digestibilities and an enhanced N retention in one trial but a reduction in a second trail. When urea and tallow were both included in the ration, absorbing urea on the grain portion of the ration improved N retention comparable to that obtained with a commercial product made of aldehyde-protected safflower meal.

In vitro rumen data showed that maximal cellulose digestion was obtained at a urea level ranging from 1.1 to 1.6% of the cellulose dry matter. When 5 or 7%

corn oil was added to the cellulose basal, the 5% oil resulted in no appreciable effect on cellulose digestibility but ammonia levels in the supernatant fluid were lower than when no fat was added. However, 7% corn oil depressed digestibility. Ammonia levels were generally higher for 7% corn oil, particularly after cellulose digestion reached a maximum. When 5 or 7% tallow was added, both levels depressed cellulose digestibility and the depression at the 7% level was greater for tallow than for corn oil. Ammonia levels were higher as the fat level increased and were higher for tallow than for corn oil.

Performance of Lambs from Ewes Fed Tallow or Protected Polyunsaturated Fat During Gestation and Lactation - D. L. Palmquist, K. E. McClure and C. F. Parker, Departments of Dairy Science and Animal Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Lambs are born with a classical clinical deficiency of essential fatty acids (1). A trail was designed to test whether this deficiency would be overcome by feeding ewes a proprietary "protected" polyunsaturated fat during gestation. The ewes were continued on the experimental diet for 28 days after lambing to further examine performance of the lambs. In addition to the experimental polyunsaturated diet, a negative control diet and a positive control diet of protected tallow were fed. The negative control (C) was corn silage treated with 1.0% urea, and supplemented with 17% dehydrated alfalfa pellets. The positive control (T) was urea-treated corn silage, alfalfa pellets and protected tallow (Alta Lipids, Boise, ID). The experimental group (P) was fed urea-treated corn silage and protected polyunsaturated fat (Alta Lipids, Boise, ID). Calculated nutrient intake during gestation met or exceeded NRC energy and protein requirements, whereas during lactation both lipid-fed groups were underfed in energy approximately 10%. Energy intake of the negative control group during lactation met NRC recommendations, as did protein intake for all groups.

Because of the limited energy intake of the fat-supplemented groups during lactation, data from single lambs only were compared. Birth weights of lambs in all groups were identical (5.35, 5.18, and 5.10 kg for C. T and P, respectively). Mean body weights of the single lambs at 28 days of age were: C, 11.8 $\pm$ 0.95; T, 10.7 $\pm$ 0.77; and P, 8.4 $\pm$ 1.32 kg. Milk production and composition of the ewes were not different at 10 and 21 days post-lambing, although milk tended to be lower and fat percentage higher in the fat-fed groups (Table 1).

Table 1. Milk Production and Composition

	C	T	P
10 Days			
No. ewes	4	5	3
Milk, g	679 ± 127	538 ± 109	548 ± 131
Fat, %	11.00 ± 1.60	11.61 ± 1.37	12.96 ± 1.65
Protein, %	6.52 ± 0.64	5.45 ± 0.55	5.91 ± 0.51
21 Days			
No. ewes	4	5	3
Milk, g	327 ± 67.0	217 ± 55.5	261 ± 90.9
Fat, %	10.37 ± 2.43	9.87 ± 2.01	15.28 ± 3.29
Protein, %	5.11 ± 0.72	5.48 ± 0.74	5.50 ± 0.93

All major fatty acids of milk fat except 4:0 and 18:3 were significantly changed by the polyunsaturated diet. P reduced 16:0 and increased 18:0, 18:1 and 18:2. Linoleate (18:2) was increased to 15.9% of the total fatty acids. The fatty acids 6:0, 8:0, 10:0, 12:0 and 14:0 were reduced by both high fat diets. T increased 18:0, 18:1, 18:2 and reduced 18:3.

Fatty acids composition of the plasma phospholipids of the lambs was changed by the diets fed the ewes. Composition of two lambs from each group at 0 (presuckling) and 1 day of age are shown in Table 2.

Linoleic acid in the plasma phospholipids of newborn lambs was not influenced by maternal diet during gestation, constituting 8% of the phospholipid fatty acids. At one day of age linoleic had increased to 14, 15, and 25% in C, T and P, respectively, and 16:1 was halved. There was no evidence that any of the lambs were deficient in essential fatty acids.

Table 2. Fatty Acid Composition of Lamb Phospholipids

	Presuckling			1 Day		
Fatty Acid	C	T	P	C	T	P
	(weight %)					
14:0	2.2	1.6	2.1	1.8	2.7	1.9
16:0	32.0	28.8	31.9	23.5	24.1	20.0
16:1	3.9	4.0	4.0	1.6	2.8	1.5
18:0	17.7	16.9	17.0	21.0	18.5	21.8
18:1	26.7	28.9	25.1	29.2	31.0	23.1
18:2	8.0	7.6	7.1	14.2	14.7	24.9
18:3	0.8	1.2	2.6	1.4	2.0	1.9
20:4	6.2	5.1	6.1	6.2	2.6	4.3

The lambs fed the experimental polyunsaturated diet did not gain as well as the other two groups. Although the poorer performance in comparison to the C group may be due in part to restricted feed intake of P ewes during lactation, this does not explain the difference between T and P, since feed intake of the ewes on these two groups was equal. Jacobson et al. (2) reported lowered growth of calves fed soybean oil.

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Plasma Fatty Acid Composition of Wethers Fed a Fat-Free Diet Intravenously - Wilson Mattos, Roger Stone, and D. L. Palmquist, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

A limited number of investigations on essential fatty acid (EFA) requirements of ruminants indicates their requirement may be unusually low. Nonruminant species, especially humans, rapidly develop signs of EFA deficiency when fed a fat-free diet intravenously.

In conjunction with a study of nitrogen balance in four sheep fed a fat-free diet intravenously, blood plasma neutral lipids and phospholipids were separated and analyzed for fatty acids composition to determine whether typical EFA-deficiency symptoms occurred.

After the implantation of the infusion catheter into the jugular vein, the animals were placed in metabolism cages and orally fed in conjunction with saline infusion for 7-10 days. At the end of this time the animals were switched from the oral diet to the intravenous diet. All animals received vitamins and minerals intramuscularly at regular intervals throughout the infusion period. Blood samples were taken prior to the infusion and at 7 day intervals.

Blood lipids were extracted with chloroform:methanol (2:1). Neutral lipids and phospholipids were separated in a salicic acid column by eluting the sample with chloroform and methanol, respectively. After saponification and methylation the methyl esters were analyzed by gas liquid chromatography.

Lauric acid (12:0), myristic acid (14:0), palmitoleic acid (16:1 n-7), increased continuously throughout the entire infusion and linoleic acid (18:2 n-6) decreased in both neutral lipid and phospholipid fractions. These changes are typical of classical EFA deficiency. However, eicosatrienoic acid (20:3 n-9) was not detected in any of the animals studied; a rise in this fatty acid is also a reflection of EFA deficiency.

By the third to fourth weeks of infusion all animals developed anemia, abnormally large numbers of immature red blood cells and fatty infiltration of bone marrow.

In conclusion, although yearling sheep intravenously infused a fat-free diet for 4-5 weeks did not show classical EPA deficiency sign of increased triene/tetraene ratio, some abnormalities were observed. These included decreased 18:2 n-6, increased 12:0, 14:0 and 16:1 n-7, and abnormal erythropoiesis.

Amino Acid Status of Ruminants Using Infusion Techniques - J. M. Asplund, Department of Animal Husbandry, University of Missouri, Columbia, Missouri.

Techniques have been developed to enable total alimentation of ruminants via infusions. The rumen is evacuated, rinsed, and filled with McDougalls saliva buffer, plastic beads, and high levels of antibiotics. These organs remain physiologically functional, but essentially non-fermentative. The animal then receives amino acids, glucose, and water soluble vitamins via a jugular catheter. Volatile fatty acids and minerals are infused directly into the rumen and fat soluble vitamins are given by intramuscular injection.

The metabolic requirement of amino acids is determined by measuring nitrogen retention and plasma free amino acids of sheep receiving different levels of the amino acid in question with all other amino acids held constant. The infusion is kept isonitrogenous by substitution of the test amino acid with glycine on an equal nitrogen basis.

Using this technique, estimations of the metabolic amino acid requirements for approximately 50-kg. wethers in grams per day for the following amino acids have estimated: methionine - 5; lysine - 6; arginine - 3; threonine - 3.

The infusion technique has also been used to determine the endogenous urinary nitrogen for sheep of this age and size. However, the absence of a fermentative rumen microflora resulted in a shifting of excretion routes for recycled nitrogen so the data did not agree with those for conventionally fed animals.

It has also been possible to determine the energy requirements by ascertaining the level of energy necessary to prevent a reduction in nitrogen balance. The data obtained in this way were in agreement with figures obtained through more conventional calorimetry.

Sulfur Amino Acid Studies in the Pre-Ruminant Calf - J. Foldager, J. T. Huber and W. G. Bergen, Departments of Dairy Science and Animal Husbandry, Michigan State University, East Lansing, Michigan.

The amino acids essential for growth in calves have not been determined but may be assumed to be qualitatively similar to those required for growth in rats. Reported requirements vary from 1.75 to 1.95% of dry matter for lysine and from .23 to .58 g per kg metabolic weight for methionine or total sulfur amino acids.

Twenty male Holstein calves were employed in a two-period changeover design with five dietary levels of methionine (1.86, 2.48, 3.10, 3.72 and 4.34 g/16 g N). All diets contained 1.05 g cystine per 16 g N. In ten two by two Latin squares two calves represented rows and two periods (9 to 15 and 21 to 27 days of age) represented columns. Milk replacer containing 17% of the total protein (18.08%) as crystalline L-amino acids was the only feed. Prepared milk (13% solids) was fed at the daily rate of 10% of body weight in two equal meals 12 hr. apart.

The response criteria were average daily gain, digestibility of dry matter and crude protein, nitrogen balance, and plasma methionine and plasma urea nitrogen levels before and 2 hr. after feeding on the first and the last day of each period. Using these methods, plus the difference between fasting and post-feeding plasma methionine levels, estimated requirements of total sulfur amino acids ranged from 3.80 to 4.00 g per 16 g N or .25 to .26 g per kg metabolic weight. Digestibility of dry matter and plasma urea nitrogen were the poorest predictors of methionine requirement. The requirement could not be estimated from plasma methionine levels before feeding or from plasma urea nitrogen on the first day of the period. Plasma methionine was the most sensitive method when poor health due to factors other than treatments were encountered. The data suggest that 3 days on the experimental feed are sufficient to estimate the amino acid requirement in calves by plasma amino acid levels. At the highest dietary level, plasma methionine 2 hr. after feeding tended toward a plateauing instead of an expected linear increase. The cause of the plateau is unknown.

Lactose Fermentation in the Cecum and the Large Intestine of the Pig - N. J. Benevenga, K. I. Kim and R. H. Grummer, Department of Meat and Animal Science, University of Wisconsin, Madison, Wisconsin.

Experiments with Chester White and Hampshire pigs revealed that the distribution of mucosal lactase in the small intestine was similar in these two breeds but that Chester White animals had 2-1/2 times more activity than Hampshires. Comparison of the mucosal lactase activity with the amount of lactose consumed revealed that the activity of the mucosa could account for only one-half of the lactose that was apparently hydrolyzed by the animal. Investigation of the lactase activity in the contents of the gastrointestinal tract revealed that the control animals had significant activity and that the activity increased in pigs fed a diet in dried whey. Calculation of the amount of lactose that could theoretically become available to the lower tract from the amount consumed and the amount retained in the stomach and small intestine, indicated that up to 50% of the lactose emptied from the stomach during the first hour after a meal could make its way to the lower intestinal tract. Anaerobic incubations of the contents from the cecum and colon without or with a 40 mM lactose as a substrate indicated that the rate of lactose hydrolysis, galactose accumulation, lactic acid accumulation, and VFA accumulation was significantly increased (150 to 200%) in animals previously fed the high whey diet. The molar percentages of acetate, propionate, and butyrate were 65, 28 and 10% respectively.

The Effect of Adequate and Inadequate Protein in the Diet for Finishing Beef Cattle - W. M. Beeson, V. M. Thomas, T. W. Perry and M. T. Mohler, Department of Animal Sciences, Purdue University, West Lafayette, Indiana.

Two feedlot trials were conducted to evaluate the effect of feeding supplemental protein for different lengths of time during the finishing period and also evaluated in the first trial was the feeding value of three types of corn grain. In the first study, steers fed no supplemental protein during the first 70 days on trial gained less (.34 kg) ($P<.01$) than steers fed protein supplement (1.30 kg). When supplemental protein was withdrawn from the steers at 70 days (weight 368 kg), daily gain (.17 kg) was less than steers fed supplemental protein for the next 84 days (1.30 kg) and feed conversion increased from 6.67 to 28.69 kg dry feed per kg gain. When steers weighed 430 kg, the removal of

supplemental protein at 140 days reduced daily gains from 1.04 to .43 kg and increased feed costs from 85¢ to $1.19 per kg of gain. Overall, cattle withdrawn from protein gained less ($P<.01$) than cattle fed supplemental protein. There was no difference in the performance of steers fed either normal, opaque-2 or waxy corn grain.

In the second study, steers withdrawn from supplemental protein at 56 days (weight 334 kg) gained less (.88 vs 1.00 kg) ($P<.05$) than a comparable group of cattle fed protein supplement continuously. Steers receiving supplemental protein for 112 and 168 days gained at essentially the same rates ($P<.10$) as cattle fed protein supplement for 56 and 228 days. Steers withdrawn from protein at 56, 112 and 168 days required more feed per unit of gain than did those steers fed protein continuously. Steers receiving a "Dehy"-vitamin-mineral supplement after protein withdrawal gained more (.98 vs .89 kg) ($P<.05$) than steers fed a corn-vitamin-mineral supplement.

Steers fed supplemental protein for 56 days had smaller loin-eye areas and lower ($P<.05$) dressing percentages than cattle fed supplemental protein for 112, 168 or for the entire 228-day feeding period. However, steers fed supplemental protein for 56 days had less ($P<.05$) fat cover and lower ($P<.10$) yield grades than steers fed protein for 112, 168 and 228 days.

Plasma urea nitrogen levels were higher ($P<.05$) at 196 days on test for steers receiving protein (12.82% protein) than steers receiving no supplemental protein (8.65% protein).

Utilization of Nitrogen from Ammonium Isobutyrate - A Grain Preservative - J. P. Fontenot, W. D. Tanner, L. F. Caswell and K. E. Webb, Jr., Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Ammonium isobutyrate has been used successfully to prevent molding of high moisture grain. Another advantage is its nitrogen content, equivalent to about 75% crude protein. Experiments were conducted to study efficiency of utilization of the nitrogen in the preservative when used alone and in combination with urea supplementation. In the first experiment nitrogen balance trials were conducted with lambs fed a low protein basal ration supplemented with 2.0 or 4.0% ammonium isobutyrate, and urea at isonitrogenous levels. Nitrogen from ammonium isobutyrate tended to be utilized more efficiently than the urea nitrogen. The only consistent difference in apparent digestibility was higher digestibility values for ether extract by the lambs fed ammonium isobutyrate. In the second experiment high moisture corn was treated with 2.0% ammonium isobutyrate or propionic acid. Nitrogen balance was studied in lambs fed rations containing high moisture corn treated with ammonium isobutyrate and propionic acid, and dry corn. In order to study the effect of combining ammonium isobutyrate-treated grain with urea, different levels of urea were fed with dry and high moisture corn. The nitrogen from ammonium isobutyrate appeared to be utilized with the same efficiency as the nitrogen from soybean meal, even when high levels of urea were fed. Feeding of ammonium isobutyrate or corn treated with this preservative resulted in higher levels of ruminal fluid isobutyric acid. There was an increase in propionic acid in ruminal fluid of lambs fed corn treated with propionic acid.

Microbial Protein Synthesis and Ruminal Turnover Rate - Cole, N. A., E. C. Prigge and F. N. Owens, Department of Animal Science and Industry, Oklahoma State University, Stillwater, Oklahoma.

Efficiency of microbial protein synthesis (MPS) was measured by abomasally sampling steers in 5 latin square metabolism studies. MPS and ruminal fluid turnover rate (TOR) were calculated based upon RNA, water and lignin content of absomasal samples. TOR ranged from 1.5 to 5.4% per hour with MPS from 6 to 27 g protein per 100 g fermented organic matter. Within trials, efficiency of MPS increased from 8 to 36% with each increase in TOR of 1%/hr, being most marked at lower TOR. Regression of MPS on TOR within trials ranged from 0.65 to 0.95. Faster TOR quantitatively increased abomasal flow of feed protein by 10% for each 1%/hr increase in TOR and increased flow of microbial protein despite decreased digestion of starch and dry matter in the rumen. Between 40 and 60% of abomasal protein across all TOR was microbial. TOR needs consideration for prediction of postruminal supply of energy and protein of ruminants.

Protein Solubility, Source and Ruminant Protein Synthesis - L. S. Bull, W. G. Helferich, T. S. Hollenshade and T. F. Sweeney, Department of Dairy Science, University of Maryland, College Park, Maryland; and Animal Science Department, University of Kentucky, Lexington, Kentucky.

Experiments have been conducted using 1000 ml. continuous culture fermentors to study the effect of protein solubility and dilution rate on ruminal parameters and metabolism. Specific variables studied were: ammonia level; net protein synthesis, and digestion of carbohydrate fractions. Net protein synthesis is here defined as the difference between trichloracetic acid (TCAA) precipitable N going into and coming out of the fermentor. The N not precipitated by the TCAA is considered nonprotein N (NPN).

Diets were comprised of (as-fed basis): corn silage, 72%, corn meal plus dicalcium phosphate, 13% and ground alfalfa hay, 5%, plus 10% of a supplement of corn gluten meal (C), soybean meal (S), Dehy 100 (D) or urea (U). Supplements were isonitrogenous and isocaloric (corn meal added). Fermentors were fed 90 or 60 g of diet in 4 meals daily, during replicated 10-day trials. Flow rate of McDougall's buffer was set to give 800 or 1600 ml flow (both used for the 90 g feeding rate, only 800 used with 60).

Net protein synthesis (% of NPN fed) for the C, S, D and U diets averaged: -7, -106, 62 and 55 for the 90 g, 800 ml flow series; 10, -16, 57 and 53 for the 90 g, 1600 ml flow series, and 33, 18, 65 and 56 for the 60 g, 800 ml flow series. Ruminal ammonia (mg % of NH_3-N) in the same order was: 2.2, 10.7, 21.9, 24.9; 1.0, 3.8, 12.6, 13.7; 1.9, 3.8, 17.6, 17.4. Cell wall digestion (%) in the same order was 48, 56, 41, 52; 26, 26, 28, 33; 26, 26, 38, 39. Lignin recovery was 100.6%. Regression of digestion of NDF on flow resulted in very little difference between source of N at high flow but an advantage for the low solubility sources at lower flow rates (when compared at equal feeding rates). Within flow rates the digestion was greater for the higher feeding rate.

(Further evaluation of the data will be made in subsequent presentations from the theses of second, third and fourth authors).

Effect of "Protected" Dietary Amino Acids on Nitrogen Balance and Plasma Amino Acids - C. R. Richardson and E. E. Hatfield, Department of Animal Science, University of Illinois, Urbana, Illinois.

Amino acids were abomasally or orally administered to growing steers or lambs to determine their effect upon nutritional status.

Studies involving abomasal infusions of amino acid(s) to growing steers were conducted to determine the first, second, and third limiting amino acids of the microbial protein of growing cattle. The steers were fed a semipurified diet containing 14.2% crude protein but essentially free of amino acids. The constant abomasal infusions provided supplemental amino acids in addition to those supplied by the microbial protein.

It was found that the supplementation of methionine decreased urinary nitrogen excretion below that of steers receiving supplemental lysine, threonine or tryptophan. In a subsequent experiment, it was observed that nitrogen retention was enhanced by the infusion of methionine plus lysine over the infusion of methionine alone.

A third study revealed that threonine was the third limiting amino acid as indicated by increased ($P<.05$) nitrogen retention when supplemented with methionine plus lysine.

Complete plasma amino acid analysis was conducted on blood samples taken four hours after the morning feeding. The infusion of methionine, lysine, threonine or tryptophan singly in the first experiment significantly ($P<.05$) increased the plasma concentration of that particular amino acid. The addition of lysine or lysine plus tryptophan to methionine, in the second study, decreased ($P<.05$) the concentration of methionine over methionine infused alone.

The data from these studies involving abomasal infusions indicated that the order of limiting amino acids in the microbial protein of growing steers is methionine, lysine and threonine.

Nitrogen balances and free amino acid concentrations in blood plasma of growing steers were used to study the efficacy of orally-administered N-hydroxymethyl-DL-methionine-Calcium (HMM-Ca) or an isomolar level of abomasally-infused L-methionine. When purified diets were fed, mean daily nitrogen retention was increased over controls by both oral HMM-Ca ($P<.075$) or abomasally-infused L-methionine ($P<.05$).

Two additional experiments were conducted to study the efficacy of HMM-Ca and/or formaldehyde (HCHO) treated soybean meal (SBM) in steers fed a natural diet. In the first study, the steers supplemented with HMM-Ca or which received the diet containing HCHO treated SBM had greater ($P<.05$) nitrogen retentions than steers which received the control diet. Treatment effects were additive. In the second study, daily gains and the gain/feed ratios of steers fed diets supplemented with HMM-Ca and/or diets which contained HCHO-treated SBM were equal to or greater than the rate and efficiency of gain of control steers.

Forty lambs were used in a study to evaluate the effects of feeding microbial resistant methionine and lysine vs. the L-isomers. When semipurified diets were fed, mean daily nitrogen retention was increased ($P<.05$) over the basal control

by oral supplementation of HMM-Ca. Moreover, nitrogen retention was increased (P .05) by feeding HMM-Ca plus di-N-hydroxymethyl-lysine-Calcium (DHML-Ca) over HMM-Ca alone. Supplementation of HMM-Ca plus DHML-Ca increased (P .05) wool growth over the basal control. Supplementation with L-methionine and L-lysine did not significantly effect biological responses.

Two chick bioassays showed that HMM-Ca and DHML-Ca are equal in efficacy to an isomolar level of DL-methionine or L-lysine, respectively.

Effects of Different Concentrations of Branched-Chain Volatile Fatty Acids and Valeric Acid on In Vitro Microbial Growth as Measured by Gas Production - A. Felix, R. M. Cook, J. T. Huber and J. W. Thomas, Michigan State University, East Lansing, Michigan.

In an in vitro trial conducted in five experiments, gas production was the parameter used to measure the effects of various concentrations of isobutyrate, 2-methylbutyrate, 3-methylbutyrate and valerate (VFA) on rumen microbial growth. Rumen fluid was collected from a fistulated Holstein cow 3 hr. after feeding. A high urea-corn silage diet was fed ad libitum for a 3 week adaptation period. The five experiments had the following culture medium: 1) rumen fluid (100 ml), Hungate buffer (200 ml), concentrate (10 g), sodium bicarbonate (.5 g), urea (.2 g), VFA (4 to 32 ul/ml rumen fluid); 2) rumen fluid (100 ml), concentrate (5 g), sodium bicarbonate (.5 g), urea (.2 g), VFA (4 to 16 ul/ml); 3) rumen fluid (100 ml), corn starch (1.0 g), ground filter paper (1.0 g), sodium bicarbonate (.5 g), urea (.1 g), VFA (4 to 16 ul/ml); 4) rumen fluid (100 ml), corn starch (1.5 g), ground filter paper (150 mg), methionine (1 mg), sodium bicarbonate (.5 g), urea (50 mg), VFA (2 to 32 ul/ml); 5) rumen fluid (100 ml), concentrate (20 g), Hungate buffer (200 ml), methionine (5 mg), bicarbonate (.5 g), urea (50 mg), VFA (4 to 28 ul/ml).

After sampling, rumen fluid was filtered through four layers of cheesecloth and kept in a water bath at 40°C. The concentrations of each of the four VFA's used varied from .5 to 8 ul/ml of rumen fluid. The number of different concentrations of the VFA mixture in each experiment which were compared with the control varied from 4 to 8. The rate of gas production was measured every 5 minutes for a period of 1 hr. using a 10-ml water lubricated glass syringe. Measurements started within 15 minutes of removing the sample from the animal. Ammonia production was determined and pH was measured before and after incubation. The changes in acetate, propionate and butyrate concentration during incubation were determined.

On the average the gas production was decreased from 19.63 to 11.28 µl/ml/min and the ammonia level slightly increased from 31.14 to 34.54 mg/100 ml of rumen fluid with the increasing concentrations of VFA mixture added to the culture medium. Compared to zero time values, the pH decreased from 6.2 to 5.4 as the VFA concentrations increased. At the highest additions of acids acetate, propionate and butyrate production was increased by 20 to 30%.

The reason for the depressing effects of VFA on gas production is not well understood. However, this trial showed clearly that VFA affected the fermentation process when added to 100 ml of rumen fluid as indicated by reduced gas production and decreased pH. Contrary to the suggestion of some workers that VFA may not show any effect on microbial growth when the concentration of the rumen fluid is higher than 20% in the culture medium, these results show that

even with about 100% rumen fluid in the medium VFA stimulated fermentation. The pH drop shows an active metabolism.

Decreased gas production might occur because there was rapid microbial growth. CO_2, which is a major component of gas produced, may have been incorporated into carbon skeletons for microbial cell synthesis. Also the utilization of carbon skeletons from VFA may decrease the protein fermentation in the medium.

PHYSIOPATHOLOGY

Dietary Components Affecting Metabolic Disorders at Parturition - W. E. Julien and H. R. Conrad, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Fifty-three dry cows at the Ohio Agricultural Research and Development Center dairy herd were placed in one of four pens for the entire dry period. All received a corn silage-concentrate ration. Pens 1 and 2 however, were maintained on an 8% crude protein intake while pens 3 and 4 received a 15% ration. Pens 1 and 3 received .65% of their dry matter as calcium and .29% as phosphorus, while pen 2 received .70% calcium and .70% phosphorus and pen 4 received .66% calcium and .65% phosphorus. Animals were bled throughout the dry period at regular intervals and within 6 hours post-freshening or prior to initiation of any therapy. It was found that in pens 3 and 4 metabolic disturbance occurred in 75% of all calvings. These included eight alert "Downer" cows, six of which died during treatment. In pens 1 and 2, the incidence of metabolic disturbance was 14% with no "Downer" cows observed. Blood constituents measured, showed no significant differences except for PUN values between groups. Individual animals studied showed no significant changes in blood constituents that would be indicative of a cause of the disorder. It was concluded that dietary protein does influence the incidence of the disease. No relationship was found to exist between the dietary mineral imbalance and clinical expression of the "Downer" conditions.

The "Downer" syndrome may be defined as follows:

1. A metabolic disturbance of the parturient dairy cow, usually terminal, occurring within a 3-week period postpartum. Clinical expression resembles hypocalcemia but can be visually differentiated.

2. It would appear to be a result of parturient changes in blood constituents. But rather it is a consequence of the nutrition input, specifically protein, fed during the dry period. Mineral imbalance in the dry cow ration, while increasing the incidence of certain disorders directly related to mineral metabolism, does not appear to be a factor in "Downer" cows.

Experimental Ovine Ruminal Acidosis - Jerome G. E. Vestweber, University of Minnesota, St. Paul, Minnesota.

Ruminal acidosis was induced in sheep by 24 hour infusion of a purified diet (corn sugar, casein, and minerals) through a rumen fistula. Twenty-four hour increases in ruminal feeding of corn sugar resulted in decreases in ruminal pH, blood pH and transketolase, while increases occurred in values for packed cell volume, blood glucose, thiamine pyrophosphate effect, blood pyruvate and

and blood L- lactate.

The onset of ruminal acidosis was associated with the development of clinical signs of central nervous system depression, ruminal stasis and tympany, salivation, congestion of mucous membranes, increased heart and respiratory rates, diarrhea, decreased urine output and lameness in all legs. The induction of ruminal acidosis in pregnant sheep did not produce abortion, cogenital abnormalities, or retained placenta.

The significant pathological lesions expressed grossly were congestion and edema of the lungs and hemorrhage of the epicardium. The kidneys were congested in the medullary region. The rumen papillae were large and erect. Significant histopathological changes were the presence of mycarditis, rumenitis and degenerative changes of the tubules and medullary congestion of the kidneys. The brain exhibited perivascular and perineuronal edema, death of neurons, gliosis and increased vascular congestion of primarily the cerebral cortex.

Evaluation of the Intravenous Administration of Xylazine Hydrochloride (Rompun) on Cardiopulmonary Function in the Bovine Species - A. P. F. da Silva, and L. L. Jackson, National Animal Disease Laboratory, Ames, Iowa.

Xylazine hydrochloride is a sedative, analgesic and muscle relaxant.

Twelve cows were administered xylazine hydrochloride intravenously at a dosage rate of .1 mg. per pound of body weight. Teflon catheters had been implanted into the saphenous artery and vein.

Blood gas determinations included PaO_2, $PaCO_2$, PvO_2, $PvCO_2$, and blood pH. The PaO_2 and PvO_2 were sharply decreased and the $PaCO_2$ and $PvCO_2$ increased. However, the results approached normal values at the recovery period. The changes were acute and clinical signs of hypoxia were not present. Blood pH was not significantly affected by xylazine hydrochloride.

The ECG records showed an increase in Q-T interval and decrease in the cardiac rate. Systolic and diastolic blood pressure were significantly decreased. The absence of apparent clinical alterations seemed to give support to the affirmation that healthy animals generally tolerate the changes caused by the administration of the drug.

Dietary Factors Affecting the Development of Polioencephalomalacia - B. E. Brent and D. A. Sapienza, Kansas State University, Manhattan, Kansas.

Polioencephalomalacia (PEM) and cerebrocortical necrosis (CCN) are synonomous terms for a central nervous disturbance of ruminants that responds to massive doses of thiamin intraveinously. Research on the problem has been hampered because only spontaneous cases were available. We accidently produced PEM while continously infusing into sheep a liquid diet based on glucose, starch and casein, with fat-soluble vitamins and minerals. (J. Animal Sci. 35:270). PEM could be produced in as little as 4 days. The rumin fluid contained high levels of thiaminase activity (J. Animal Sci. 35:1134). Thiaminase I (EC. 2.5.1.2.) carries out substitution reactions in conjunction with several possible co-substrates, giving rise to several possible thiamin antimetabolites. Thiaminase II simply hydrolyzes thiamin at the methylene bridge. Because of the rapidity of PEM development in our studies, thiaminase I seems more likely to be involved.

Although thiamin is presumably synthesized in the rumen fluid thiamin is much lower on high concentrate diets than on roughage diets (J. Animal Sci. 39:251).

During rapid changes from alfalfa to ground milo, lactic acid and thiaminase increased in the rumen (J. Animal Sci. 39:251). Since the optimum pH for thiaminase I is 5, and since histamine, which accumlates during lactic acidosis, is a possible thiaminase I co-substrate, a correlation between PEM and ruminant lactic acidosis seems likely.

Recently, several thiamin responsive cases of PEM have developed on wintering rations when self-fed supplement intake was controlled by gypsum. This leads to a possible interaction between PEM and sulfate, which should be examined.

Some Evidence that Rumen Acidosis is an Inadequate Term for Grain Engorgement in Ruminants - R. W. Dougherty, Ames, Iowa.

Much of the experimental and clinical work on grain engorgement has been directed to changes in the ruminoreticulum of cattle and sheep. Recent studies have shown that many changes occur in the abomasum, small intestine and cecum. These changes may influence the signs occurring in grain engorged ruminants.

It is strongly recommended that researchers working with this disease should review, carefully, the ten reports of Turner and Hodgetts in Australia that can be found in the Commonwealth Scientific and Industrial Research Organization's bulletin, Melbourne, Australia, 1949-1959. These are classical works and if read carefully give evidence that factors other than acidosis may be involved in the syndrome.

Evidence was presented, using the Limulus amebocyte lysate assay (LAL assay), that endotoxin was present in the blood of experimentally grain engorged sheep.

This latter work was preliminary and recent improvements in the LAL assay should encourage additional experimental work along these lines.

It is proposed that the term "rumen acidosis" be replaced with the more inclusive term, "grain or high-energy ration overload."

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