Reports

Dissimilation of Alfalfa Saponins by Rumen Bacteria

Experimental symptoms of bloat have been produced in sheep by the oral and intravenous administration of alfalfa saponins, demonstrating that saponins have more than one mode of action (*1*). However, the fate of the saponins, the action of microorganisms on them, and the mechanism in the production of bloat are still not known. Recently, Hungate *et al.* (*2*) and Jacobson and Lindahl (*3*) postulated that polysaccharide slime production by ruminal microorganisms might contribute to a stable froth formation which interferes with the normal eructation of fermentation gases in ruminants. The present report presents evidence that alfalfa saponins are utilized by certain rumen bacteria, with resultant production of acids, gas, and large amounts of slime.

Saponin-digesting bacteria were isolated from the rumens of six steers that were being fed a fresh alfalfa diet, by means of modifications of techniques described previously (*4*). Rumen contents were inoculated into two parallel dilution series of 20-percent-rumen-fluid agar shake-tubes, and one series was enriched with 0.5 percent alfalfa saponin. Organisms utilizing saponin were detected by the larger size of the colony as compared with that of the nonsaponin control. Organisms presumed to be saponin bacteria were observed on dilution series from all six animals, with a peak number of 680 million per milliliter of ruminal fluid. Microscopic examination of different colonies showed cells of variable morphology; the predominant type was a small, Gram-negative, curved, motile rod.

Pure cultures of several strains of the bacteria were grown in order to study their characteristics and to obtain quantitative evidence of saponin dissimilation from the chemical analyses of metabolic end products. Fermentation products from the breakdown of 200 mg of alfalfa saponins (*5*) by a representative strain in a yeast-extract peptone medium yielded (in millimoles): carbon dioxide, 1.9; acetic acid, 0.23; butyric acid, 0.15; formic acid, 0.52; lactic acid, 0.14; and traces of ethanol and propionic acid. The organism had a fermentation pattern similar to the atypical strains of *Butyrivibrio* described by Bryant (*6*). Large amounts of slime from the fermentation of saponins were harvested by centrifugation at the end of the incubation period, while scant growth occurred in control medium without saponin.

In the various experiments the dry-weight yield of viscous slime material was approximately 50 percent of the saponin provided as substrate. When the organism was grown on glucose, 10 percent of the substrate appeared as cellular material. Acid hydrolysis of the slime matter gave copious amounts of copper-reducing compounds, indicating its polysaccharide or other glycosidic nature. Heavy froth formation from the microbial degradation of saponins was readily demonstrated in rumen contents containing viable microorganisms, but froth was not produced from the saponins when the bacteria and protozoa were inactivated by mild heat. Rapid gas evolution formed a frothy foam layer 40 mm above the liquid phase 3 hours after 2-percent saponin was administered to rumen ingesta.

Our results support the theory that "frothy bloat" of ruminants maintained on rich forages, such as clover and alfalfa, may be caused by an increase in the production of bacterial polysaccharide slime. The latter forms stable foam in which the rumen fermentation gases are entrapped in the ingesta as numerous small gas bubbles and are prevented from escaping (*2*). In manometric experiments, Hungate (*2*) has observed a correlation between the foam production of rumen ingesta and the intensity of the bloat symptoms of cattle.

The evidence of a rapid microbial decomposition of legume saponins, with the concomitant production of gas and slime, indicates the interaction which exists between the saponins and certain rumen bacteria and demonstrates how these plant compounds may contribute to bloat. In addition, bacterial attack on soluble sugars in the plant materials probably enhances the quantity of slime in the rumen. It is noteworthy that Barrentine (*7*) has had some success in the control of bloat in cattle on rich pasturage through the administration of antibiotics.

JOSE GUTIERREZ
R. E. DAVIS
I. L. LINDAHL
Animal Husbandry Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

References and Notes

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5. The composite alfalfa saponins were furnished through the courtesy of Western Utilization Research Branch and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif.
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Spectral Changes Accompanying Binding of Acridine Orange by Polyadenylic Acid

A recent article by Szent-Györgi and co-workers has cited evidence for formation of a complex between the fluorescent dye acridine orange and the adenine portion of adenosine triphosphate, as well as adenine itself (*1*). In view of this result, it was of interest to examine the interaction of this dye with synthetic polyribonucleotides. This has been done for polyadenylic acid with somewhat unexpected results.

Polyadenylic acid was prepared by the action upon adenosine diphosphate of the nucleotide polymerizing enzyme isolated from *Micrococcus lysodeikticus* by a method described elsewhere (*2*). Spectral and fluorescence studies were confined to concentrations of acridine orange sufficiently low ($< 5 \times 10^{-5} M$) so that the monomer-dimer equilibrium, as studied by Zanker (*3*), is displaced greatly in favor of the monomer. No spectral effect was observed upon addition of adenosine monophosphate. However, a quite marked effect was observed

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