Effect of Dietary Extremes on Impala (Aepyceros melampus) Rumen Epimural Flora

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The impala, classified as an intermediate feeder among wild ruminants, grazes exclusively on green grasses during the summer rains but turns to an essentially browse habit during the winter, when a diet of leaves, pods, and twigs is supplemented with dried grasses. Scanning electron microscope studies of bacteria attached to the rumen epithelia of animals feeding on digestible, carbohydrate-rich grazing diet showed a high level of surface colonization, with pockets of mixed morphological types occupying spaces vacated by desquamated epithelial cells. Rumen wall colonization in browsing animals on an indigestible, abrasive, low-plane diet showed denudation of distal papillar surfaces with sparse homogeneous adherent flora in the microprotected areas towards the bases. Forage characteristics associated with raised volatile fatty acid concentrations in ingesta, higher rate of epithelial sloughing, and minimum abrasion were interrelated with density and complexity of the adherent bacterial flora.

Studies on the bacterial flora associated with rumen epithelia have indicated a diversity of form, function, and distribution, subject to certain environmental influences (1, 4, 5, 14, 15). Although many members of the microflora colonizing the squamous epithelium of the sheep rumen are those already isolated from lumen contents (4, 15), there would also appear to be some intimately adherent bacteria not yet recovered in vitro. Urease activity has been shown to be a function associated with this flora (14) which occupies a niche of maximum urea concentration at the rumen wall. Colonization of desquamating cells may also indicate proteolytic digestion of dead squames (5) with a possible influence on mitotic activity and transit time of the epithelia. Variations in chemical and physical structure of the ingesta have also been shown to exert a strong influence on the distribution of adherent rumen bacteria. Rumen ingesta in cattle stratifies into fluid, fibrous mat, and dorsal gas space. The epithelial microenvironment of the latter supports maximum colonization by adherent bacteria in contrast to the denudation of exposed papillae by physical abrasion of the fibrous mat. Disparity demonstrated in the distribution, if not in total numbers, of adherent bacteria in animals on high- and low-plane diets, was attributed to physical characteristics of the ingesta rather than chemical composition (14).

To study the influence of diet on bacteria attached to rumen epithelia, gross nutritional extremes, beyond those normally used in feeding trials in domestic animals, should be sought. Dinsdale et al. (5) successfully used the approach of infusion feeding to remove the physical effects of diet altogether and to show that an epimural flora with proteolytic function is still retained. An alternative approach is to use an animal species with the ability to adapt to cyclical extremes of forage quality. The impala (Aepyceros melampus) is a medium-sized antelope, ubiquitous in Africa, with the rare propensity for grazing on succulent green grasses during the summer rains but turning to browse on dried leaves and pods in the winter drought. The impala is described as an intermediate feeder (8) with rumen anatomy not dissimilar to that of domestic animals. Saccular subdivision and surface distribution of papillae are characteristic of bulk-roughage eaters but there is an extension of rumen surface area in the form of longer, denser papillae when the animal turns to "juicy concentrated herbage."

In this study, simple rumen function parameters as influenced by the dietary extremes of the impala are related to differences in the epimural flora seen by scanning electron microscopy.

**MATERIALS AND METHODS**

Sources of material. Two adult male and one young male impala were sampled during the wet season in December from Pilansberg game reserve (25°15'S, 27°00'E). Their habitat was Combretum imberbe open savanna interspersed with termittaria thickets. The grass cover was heterogeneous, with Cymbopogons, Urelytrum, and Brachiaria predominating. An adult male and young female were collected at Nylsvley game reserve (24°40'S, 28°40'E) in June, during the winter drought. The vegetation was mainly Burkea africana, Eragrostis, Digitaria savanna with patches...
TABLE 1. VFA in impala rumen fluid

<table>
<thead>
<tr>
<th>Determination for:</th>
<th>Total acids (mmol/liter)</th>
<th>Acetic (molar %)</th>
<th>Propanoic (molar %)</th>
<th>Butanoic (molar %)</th>
<th>Methyl propanoic (molar %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Browser</td>
<td>127.0 ± 4.0</td>
<td>76.5 ± 2.1</td>
<td>16.5 ± 0.7</td>
<td>6.0 ± 1.4</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Grazer</td>
<td>168.0 ± 16.0</td>
<td>68.6 ± 5.0</td>
<td>19.6 ± 3.2</td>
<td>9.6 ± 2.1</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

Significance $P = 0.05$ NS$^a$ NS NS NS NS

$^a$ NS, Not significant.

FIG. 1. Papillar surface of browsing impala rumen with convoluted surface and attached squames. Bar = 10 µm.

FIG. 2. Papillar surface of grazing impala rumen showing smooth surface and cell-shaped pockets. Bar = 10 µm.

of *Acacia* thorn savanna on sites of abandoned villages. All animals were culled in the early evening, between 6:00 p.m. and 11:00 p.m.

**Rumen acidity.** Rumen acidity was measured with a Knick Portatest pH meter. Six random readings were taken throughout the rumen-recticum contents immediately after the animal was eviscerated.

**VFAs.** Rumen contents were well mixed by hand before sampling into stoppered bottles. These were chilled on ice for approximately 4 h before transfer to $-20^\circ$C for storage. All analyses were done in duplicate on a Pye GCD gas chromatograph fitted with F.I.D., using Chromosorb 101 porous polymer column packing (Johns-Manville) run isothermally at 215°C (3). Although culture fluids for taxonomic gas chromatography were passed through a Dowex 50-W cationic exchange column before injection, rumen fluids were merely clarified by centrifugation, a step that avoids quantitative inaccuracy at the expense of column longevity.

**Scanning electron microscopy.** Blocks (1 cm$^2$) of rumen wall were excised within minutes of evisceration, washed vigorously in multiple changes of cold isotonic 0.1 M cacodylate buffer (pH 7.0) until visibly free of adherent material, and fixed in 1.5% glutaraldehyde in the same buffer. Sampling sites, chosen specifically to compare colonization on papillae of different lengths, were midline and lateral aspects of both the anterior and posterior dorsal sacs. Linear groups of six to eight papillae were dissected out for measurement and postfixation in 1% osmium tetroxide. Tissues were dehydrated, critical point-dried in carbon dioxide, coated with 20 nm of gold, and examined in a Jeol T20 instrument at 19 kV.

**RESULTS**

Rumen contents of all summer-grazing animals consisted of a rich, odiferous sludge of green grasses with a few forb fragments. In
contrast, the impala rumen contents in midwinter reflected a heterogeneous forage selection of approximately equal proportions of dry grasses and browse diet made up of dried dicotyledonous leaf fragments, seed pods, and even small twigs. Previous reports on rumen content and winter feeding habits of impala have described similar diet composition in far greater detail (7, 8, 11). Suspension of culling operations during the summer breeding season has made rumen analysis data on grazing impala virtually unobtainable, but a concurrent study on forage selection by observation confirms up to 95% grass intake at this time of year (S. Cooper; unpublished data). The pH values recorded from random readings of unmixed fresh rumen contents in situ were in the range 5.7 to 6.3 for the three grazing animals and between 6.5 and 6.8 for the browsers. As with other ruminants, there was slight variation within different compartments of the same rumen. Volatile fatty acid (VFA) analyses on well-mixed ingesta samples are shown in Table 1. The difference in total acid concentration between the two groups was significant but although the contribution of butanoic and propionic acids to the VFA pool in animals on high-plane rations has been well documented (9), this is shown here as a nonsignificant trend. As detailed comparative analysis of papillar structure and distribution was not anticipated in this study, sampling was limited to blocks excised from similar sampling sites in each rumen. The epithelial surface of the grazing impala was raised into slender papillae up to 11 mm long in the anterior dorsal sac and 4 mm long on the posterior dorsal sac. The surfaces were smooth with numerous cell-shaped pockets similar to those described in sheep (15) (Fig. 2). Papillae in the browser’s rumen were much shorter, with a maximum length of 6 mm, the surfaces of which were roughened and convoluted. Cell-shaped pockets were rarely seen and were confined to the bases of papillae (Fig. 1). The bacterial flora associated with the rumen epithelium of the two groups of animals showed wide variation in distribution and form. An attempt was made to quantitate the colonization patterns by using a grading system of 1 to 4, corresponding to the area covered by bacteria, expressed as a percentage, in accordance with McCowan et al. (14). It was found, however, that the colonization was so variable from cell to cell, and even between adjacent papillae, that the grading system could only be used to illustrate gross differences, such as was found between the two groups of animals, rather than subtle variations that may be present from site to site or between individual animals within the group. The bacterial cover of papillae of the impala on a browse diet was never more than 50% of areas, confined to the proximal protected surfaces. Tips of papillae tended to be poorly colonized, with less than 25% coverage seen in troughs and folds of the convoluted epithelium (Fig. 3). The flora was simple, made up of pure or mixed microcolonies of rods and cocci (Fig. 4). Quite a different

FIG. 3. Surface of Fig. 1 with sparse bacterial cover, even under loose squame. Bar = 10 μm.

FIG. 4. Simple microflora made up of rods and cocci found on a browser’s rumen wall. Bar = 1 μm.
picture was shown by all of the animals in the grazing group. The rumen papillae carried a very high attached bacterial burden, often covering 100% of the surface at the bases, lessening to between 50 and 75% towards the tips (Fig. 5). Superficial epithelial cells were covered with rods and cocci, with considerable variation from cell to cell. Each pocket was densely colonized by a complex microflora of mixed morphological types, but the composition was not always the same. A useful marker to illustrate this observation was the presence of treponemal spirochetes (Fig. 6). Three or four different types of spirals were seen in some pockets, yet none at all were seen in others. Thus it is probable that the anonymous rod, curved, and coccal forms also varied from niche to niche. Although borrelian spirochetes were seen in stained films of impala rumen contents, epithelial attachment by these organisms was not observed.

**DISCUSSION**

The unusual feeding habit of the impala has afforded a unique opportunity to study some parameters that may affect the epimural bacteri­al flora in the complex ecosystem of the rumen. The heavy bacterial burden adherent to the rumen wall of summer-grazing animals was associated with a soft, digestible diet and raised VFA concentrations. The heavily colonized pockets in the papillary epithelium are probably spaces left by desquamated cells, indicating a high epithelial cell turnover. The change to a more lignified, indigestible, carbohydrate-poor forage in winter brought about a drop in VFA concentration, longer transit time of superficial squames, and sparse colonization by adherent bacteria. Sampling free-range wild animals has unfortunate restrictions that have to be considered in the interpretation of results obtained. Marked diurnal changes in rumen parameters which are closely linked to feeding habits are usually unknown to the experimenter. In tame wildebeest there is a decline in VFA concentrations during the day (10), indicating that consistency of sampling times is imperative in any comparative study. Although the preceding forage and water intakes of the animals in this study were unknown, they were all sampled within a 5-h period in early evening, to some extent minimizing this variable. Differences shown in total acid concentrations associated with dissimilar diets can be considered significant with lower values found in winter-browsing animals. Compacted rumen contents in both groups of animals, suggesting adaption to a water limiting environment, would not only influence VFA concentration in the fluid phase but also exert an overall abrasive action comparable to the limited effects of the fibrous mat encountered in the stratified rumen contents of cattle (14). This action would be expected to be more pronounced with the heterogeneous, lignified ingesta from the winter diet of the impala.

Although limited facilities in the field made
attempts at quantitative culture counts somewhat unreliable in terms of absolute numbers, the range of microorganisms recovered in roll-tube culture, identified to generic level, was similar to that found in other ruminants (unpublished data). Concurrent work in this laboratory and reports of other workers (4, 15) highlight the difficulty in culturing all members of bacterial populations adherent to epithelial surfaces. This is illustrated by the different types of spirochetes seen in the scanning electron microscope but which failed to grow in anaerobic culture media. Total counts of bacteria in rumen fluid would therefore have little relevance in studies of the epimural flora; only a proportion of those strains that can be cultured are likely to be potentially adherent and of those that do adhere, some cannot be cultured. Of more pressing interest is the study of individual adherent strains in the rumen to see whether they obey rules similar to those formulated for other microenvironments. Colonization of mucous membranes of the oral cavity is dependent not only on innate affinity, but also on a sufficiently high population density of potentially adherent bacteria in saliva (6). If these principles apply to the rumen then one would expect shifts in the epimural flora similar to, but independent of, the suspended bacterial populations when the host animal is subject to dietary changes (12). Observations on the impala indicate this may be the case as the browser rumen showed fewer adhering bacteria of limited morphological diversity in comparison to their grazing counterparts.

Little can be deduced from this study on the role of epithelial exfoliation in rumen wall colonization. The interpretation by Mead and Jones (15) that the diamond-shaped pits on sheep rumen papillae are holes left by desquamated cells can also be applied to similar pits on the long slender papillae of the rumens of impala on high-energy forage. That they were not seen on the short papillae in the browser would indicate a relative hypoplasia, but it would be an oversimplification to attribute the difference to any single factor, such as an abrasive diet, reduced bacterial colonization, or changed VFA levels.

In conclusion, this study has suggested that the morphology and distribution of bacteria attached to the rumen wall in the impala model are multifactorial phenomena subject, in part, to cyclical changes in diet structure.

ACKNOWLEDGMENTS

Collection of scientific data on free-living wild animals is dependent on carefully controlled culling operations normally taking place out of breeding season. Access to impala in midsummer could only have been achieved through integration of a research program with culling schedules, and in this regard I thank the Transvaal and Bophuthatswana Nature Conservation Departments for their assistance.

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LITERATURE CITED


