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# Bacterial and Fungal Numbers in Ruminal and Cecal Contents of the Blue Duiker (*Cephalophus monticola*)†

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**Total and cellulolytic bacterial and fungal numbers were determined in ruminal and cecal contents of 20 blue duikers (*Cephalophus monticola*). The animals were equally divided by sex and fed two diets, either high roughage or high concentrate. The mean concentration for total bacterial numbers in the rumen was 26.0 × $10^8$/g of contents, with values ranging from 2 × $10^8$/g to 93 × $10^8$/g. Cellulolytic numbers averaged 6.0 × $10^8$/g with a range of 1.5 × $10^8$/g to 24.0 × $10^8$/g. No differences related to sex or diet were found. In contrast, total bacterial numbers in the cecum differed between diets ($P < 0.02$), i.e., 1,046 × $10^6$ bacteria per g for animals fed the high-forage diet compared with 166 × $10^6$/g for those fed the high-concentrate diet. Cellulolytic bacterial counts in the cecal contents averaged 3.1 and 7.0% of the total counts for the high-forage and high-concentrate diets, respectively. Low concentrations of fungi were found in both ruminal and cecal contents of some, but not all, animals. Unexpectedly, concentrations of bacteria and fungi in the rumen and cecum were highly correlated with their total numbers (concentration multiplied by total weight of contents).**

The blue duiker (*Cephalophus monticola*), which weighs 4 to 6 kg at maturity, is probably one of the smallest ruminants in the world. It is native to central and southern Africa and appears to feed on grasses, immature leaves, and fruit. A small number of these animals was brought to The Penn State University in 1982; however, enough animals for this type of survey were not available until recently. The present study reports bacterial and fungal numbers in the ruminal and cecal contents of duikers fed a high-roughage or high-concentrate diet.

## MATERIALS AND METHODS

**Animals.** Twenty blue duikers (*C. monticola*), 10 males and 10 females, were used in this study. Four animals were brought to the autopsy room each morning from the housing facility. A veterinarian administered a tranquilizer; and the duikers were killed by lethal injection. Ruminal and cecal contents were collected upon autopsy.

**Diets.** Two pelleted diets, a high-roughage diet (57.2% neutral detergent fiber) and a high-concentrate diet (26.8% neutral detergent fiber), were each fed to 10 blue duikers, five males and five females. The diets were administered at 90% of ad libitum intake for at least two months prior to killing of the animals. The compositions of the diets are given in Table 1.

**Enumeration of bacteria.** Total and cellulolytic bacterial numbers were estimated simultaneously in the same medium by using the most-probable-number (MPN) procedure described by Dehority et al. (7). Ruminal and cecal contents were diluted as described previously (5, 10). After 14 days of incubation, cellulolytic bacterial numbers were estimated by visual loss of cellulose and total bacterial numbers were estimated on the basis of a decrease in medium pH.

**Enumeration of fungi.** Both total and cellulolytic fungal numbers in ruminal and cecal contents were also determined with an MPN procedure. The procedure has been described by Obispo (20) and is similar to the MPN procedure of Dehority et al. (7). The composition of the basal medium (7) was changed by adding 0.2% Trypticase–0.05% yeast extract–0.45 ml of VFA mixture (3) per 100 ml of medium and decreasing the amount of ruminal fluid to 20%. A filter-sterilized antibiotic solution was added before use to inhibit bacterial growth. Final antibiotic concentrations in the medium were 2,000 U of penicillin per ml and 130 U of streptomycin per ml.

**Statistical analysis.** Data were analyzed by Harvey least-squares analysis of variance (11).

## RESULTS

The total and cellulolytic bacterial numbers in the ruminal contents of blue duikers are presented in Table 2. Since neither concentration, total numbers, nor the percentage of the population which was cellulolytic was affected by diet or sex, the data were compiled as overall means. Table 3 presents the same parameters for cecal contents. Both concentration and total numbers of bacteria were significantly higher ($P < 0.02$) in cecal contents of duikers fed the high-forage diet.

Fungal numbers in ruminal contents were not affected by diet or sex (Table 4). However, the percentage of fungi which were cellulolytic was significantly higher ($P < 0.05$) in males than in females. No cellulolytic fungi were found in female duikers.

Cecal contents contained very low numbers of fungi, averaging less than 10% of the concentration found in the rumina (Table 5). No significant differences were associated with diet or sex. It should be pointed out that only one animal, a male fed the high-concentrate diet, harbored any cellulolytic fungi in its cecum.

The sporadic occurrence and low numbers of fungi in ruminal and cecal contents raised a question about the adequacy of the medium. After the fungal assays had been

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TABLE 1. Compositions of pelleted diets fed to blue duikers

| Ingredient | % of diet[a] | |
|---|---|---|
| | High concentrate | High forage |
| Alfalfa meal | 25 | 50 |
| Ground oats | 20 | 5 |
| Ground corn | 18.25 | 2.25 |
| Wheat middlings | 15 | 25 |
| Soybean meal | 7.75 | 6.75 |
| Molasses | 7.5 | 4.0 |
| Distiller's grains with solubles | | 2.5 |
| Meat-poultry byproduct | 5 | |
| Salt | 0.3 | 0.3 |
| Sodium bicarbonate | 0.8 | |
| Vitamin premix[b] | 0.39 | 0.8 |
| Limestone | | 0.25 |
| Lignin binder | | 3.0 |

[a] Dry matter basis.
[b] Vitamin E-20000, 0.15%; trace minerals, 0.1%; Se, 0.05%; vitamin A-30000, 0.03%; vitamin $D_3$ 6M, 0.01%; Tylan 10, 0.05%.

TABLE 3. Total and cellulolytic bacterial numbers in cecal contents of blue duikers

| Diet and value | Concn ($10^6$/g) | | No.[a] ($10^8$) | | % Cellulolytic bacteria |
|---|---|---|---|---|---|
| | Total | Cellulolytic[b] | Total | Cellulolytic | |
| High forage | | | | | |
| Mean[c] | 1,046.3[d] | 25.2 | 238.1[d] | 5.1 | 3.1 |
| Range | 93–2,400 | 0–240 | 30–634 | 0–48 | 0–26.7 |
| High concentrate | | | | | |
| Mean[c] | 165.6[e] | 1.7 | 37.5[e] | 0.5 | 7.0 |
| Range | 7–430 | 0–9 | 3–111 | 0–3 | 0–58.1 |
| SE | 232.2 | 17.0 | 50.4 | 3.4 | 4.4 |

[a] Concentration multiplied by total weight of cecal contents.
[b] Cellulolytic bacteria were not detected in four animals, one male and one female, fed each diet.
[c] Ten animals, five males and five females, were used.
[d,e] Means in the same column followed by different superscript letters differ significantly ($P < 0.02$).

incubated for at least 10 days, six MPN tubes from the highest dilution ($10^{-6}$), one from each of six animals, were inoculated with a $10^{-2}$ dilution of cattle ruminal contents. All tubes showed marked fungal growth after several days, indicating that the medium was satisfactory.

The relationship between total numbers of bacteria which occur in the rumen and cecum and their concentration per gram of contents are shown in Fig. 1. Correlation coefficients for these data were 0.93 and 0.97 for the rumen and cecum, respectively. Similar correlations were calculated with the fungal data, resulting in coefficients of 0.96 for the rumen and 0.98 for the cecum. The weight of ruminal or cecal contents was poorly correlated with bacterial concentrations ($r = 0.15$ for the rumen and $r = -0.34$ for the cecum).

## DISCUSSION

In a recent study, we reported ruminal bacterial concentrations from domestic cattle and sheep determined by using these same MPN procedures (7). On the basis of data from 12 animals (8 calves and 4 sheep), total and cellulolytic bacterial numbers per gram of ruminal contents averaged $15.12 \times 10^9$ and $7.46 \times 10^8$, respectively, for animals fed either a high-roughage or high-concentrate diet. The mean concentration of total ruminal bacteria was considerably lower in the blue duikers, whereas the concentration of cellulolytic organisms was much closer to the numbers observed in domestic ruminants.

Cowan et al. (4) compared the nutrient digestibilities of rabbit pellets, alfalfa hay, and fresh frozen alfalfa by blue duikers and sheep. They found that dry matter and neutral detergent fiber digestibilities were significantly lower ($P < 0.05$) in duikers; however, acid detergent fiber digestibilities were similar for the rabbit pellet and alfalfa hay diets. The bacterial concentrations observed in the present study appear to reflect these digestibilities, i.e., lower concentrations of total bacteria and similar concentrations of cellulolytic organisms.

Concentrations of viable bacteria in the ruminal contents of both domestic and wild ruminants vary considerably, ranging from about $3 \times 10^7$/g to $300 \times 10^9$/g for animals fed a wide range of diets (8, 14, 17, 21, 22, 24, 26). Cellulolytic numbers are much lower, ranging from $4 \times 10^5$/g to $1.1 \times 10^9$/g. The present data fall within these reported ranges. Although blue duikers have not been studied previously, Giesecke and van Gylswyk (8) have reported viable counts of ruminal bacteria from wild impalas and springboks, both of which are in the same subfamily as blue duikers (*Antilopinae*). Total and cellulolytic counts per gram of ruminal contents were $23 \times 10^8$/g and $0.94 \times 10^8$/g for impalas and $55 \times 10^8$/g and $1.1 \times 10^8$/g for springboks. Although total bacterial concentrations in blue duikers are fairly similar, cellulolytic bacterial numbers are considerably higher. This may be a reflection of the higher quality of the fiber in the

TABLE 2. Total and cellulolytic bacterial numbers in ruminal contents of blue duikers

| Value | Concn ($10^8$/g) | | No.[a] ($10^{10}$) | | % Cellulolytic bacteria |
|---|---|---|---|---|---|
| | Total | Cellulolytic | Total | Cellulolytic | |
| Mean[b] | 26.0 | 6.0 | 68.2 | 16.5 | 33.1 |
| Range | 2–93 | 1.5–24 | 4–312 | 1–55 | 6–100 |
| SE | 5.3 | 1.2 | 15.8 | 3.5 | 6.3 |

[a] Concentration multiplied by total weight of ruminal contents.
[b] Twenty animals, 10 males and 10 females, were used. Five animals of each sex were fed a high-roughage diet, and five received a high-concentrate diet.

TABLE 4. Total and cellulolytic fungal numbers in ruminal contents of blue duikers

| Value | Concn ($10^2$/g) | | No.[a] ($10^4$) | | % Cellulolytic fungi[b] | |
|---|---|---|---|---|---|---|
| | Total[c] | Cellulolytic[d] | Total | Cellulolytic | Male | Female |
| Mean[e] | 32.5 | 25.1 | 53.2 | 33.9 | 33.5 | 0 |
| Range | 0–350 | 0–350 | 0–481 | 0–481 | 0–100 | 0 |
| SE | 17.7 | 17.2 | 26.4 | 23.5 | 10.7 | 10.7 |

[a] Concentration multiplied by total weight of ruminal contents.
[b] The difference ($P < 0.05$) between males and females was significant. There were 10 animals in each group. Five animals of each sex were fed a high-roughage diet, and five received a high-concentrate diet.
[c] Fungi were detected in six males and four females.
[d] Cellulolytic fungi were present in only four males.
[e] The data are based on 20 animals, 10 males and 10 females, except for those under % cellulolytic fungi.

TABLE 5. Total and cellulolytic fungal numbers in cecal contents of blue duikers

| Value | Concn (per g) | | No.[a] ($10^2$) | | % Cellulolytic fungi |
|---|---|---|---|---|---|
| | Total[b] | Cellulolytic[c] | Total | Cellulolytic | |
| Mean[d] | 230 | 5.5 | 61.2 | 10.6 | 1.9 |
| Range | 0–930 | 0–110 | 0–274 | 0–13 | 0–38 |
| SE | 60.4 | 5.5 | 17.0 | 0.6 | 1.9 |

[a] Concentration multiplied by total weight of cecal contents.
[b] Fungi were detected in nine males and nine females.
[c] Only one animal, a male, contained cellulolytic fungi, at a concentration of 110/g.
[d] Twenty animals, 10 males and 10 females, were used. Five animals of each sex were fed a high-roughage diet, and five received a high-concentrate diet.

diets fed to the duikers compared with that of animals in the wild.

Information on cecal bacteria in ruminants is limited. Four studies have reported concentrations of total viable bacteria ranging from $4.3 \times 10^5$/g to $15.8 \times 10^8$/g (13, 16, 19, 25). Cellulolytic numbers, reported in only two studies, varied between $6 \times 10^6$ and $1 \times 10^8$ (13, 16). Lewis and Dehority (15) measured viable total and cellulolytic bacterial concentrations in sheep fed 100% orchardgrass hay, 60% cracked corn–40% orchardgrass hay, or 80% cracked corn–20% orchardgrass hay. The concentrations were $6.3 \times 10^8$/g, $497.4 \times 10^8$/g, and $496.0 \times 10^8$/g of cecal contents for total viable bacteria and $1.2 \times 10^6$/g, $0.6 \times 10^6$/g, and $0.4 \times 10^6$/g for cellulolytic bacteria, respectively. The increase in total bacterial numbers resulting from addition of corn to the diet, either 60 or 80%, was significant ($P < 0.01$). The present results for total and cellulolytic bacterial concentrations in the ceca of high-forage diet-fed duikers, $10.5 \times 10^8$/g and $25 \times 10^6$/g, are within what appears to be the normal ranges for forage-fed ruminants. However, total numbers were decreased by 85% in the high-concentrate diet-fed duikers compared with a 78-fold increase from feeding of concentrates in the study of Lewis and Dehority (15). Although the concentration of cellulolytic organisms was higher in the duikers, it followed a similar pattern by decreasing when concentrates were added to the diet. In both studies, animals were fed at 90% of ad libitum intake.

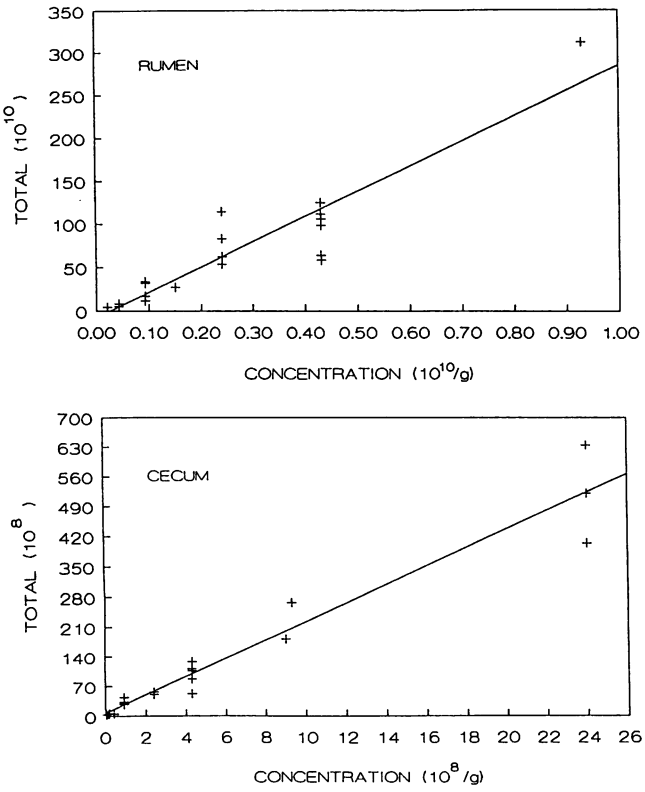


FIG. 1. Regression of total bacterial numbers in rumina and ceca (concentration times weight of contents) upon bacterial concentrations.

In ruminants, feeding of concentrates (15) or soluble carbohydrates (16) slightly lowered the cecal pH, but all reported values are between pHs 6 and 7. One might speculate that in the study by Lewis and Dehority (15), the increased concentration of bacteria in cecal contents which resulted from feeding of corn was probably caused by the availability of more energy in the cecum. Unfortunately, they did not report ruminal pH values. In the present study, the mean ruminal and cecal pH values were essentially the same for both diets, i.e., 6.2 and 8.0, respectively. These data offer little insight into the significant decrease in cecal bacterial concentrations caused by feeding with concentrates.

Fungal concentrations in ruminal contents from the duikers were not affected by diet; this appears to agree with previous studies of cattle and sheep. Reported values range from 64/g or ml to 44,000/g or ml (1, 9, 12, 23), which is fairly similar to the range of 0 to 35,000/g in the present study. Akin et al. (1) reported cellulolytic fungal concentrations ranging from 36/g to 7,360/g, constituting 5 to 93% of the total population. Only four duikers, all males, contained cellulolytic fungi, with concentrations of 90/g, 150/g, 15,000/g, and 35,000/g, which constituted 100, 35, 100, and 100%, respectively, of the total fungi. The animal containing 90 fungi per g of ruminal contents was fed the high-roughage diet, and the other three received the high-concentrate diet. The significant difference between sexes in percent cellulolytic organisms appears to be due to chance.

Fungi were detected in cecal contents of 18 duikers, nine males and nine females, with concentrations ranging from 40/g to 930/g. These values are similar to those reported by Grenet et al. (9) for cows fed a variety of diets, i.e., 70/g to 1,933/g. Only one animal, a male, contained cellulolytic fungi, and they constituted 38% of the total fungi. It is interesting that fungi occurred in the cecal contents of nine animals that were apparently devoid of ruminal fungi (less than 4/g). One of the animals without cecal fungi did contain 15,000 fungi per g of ruminal contents.

Bauchop (2) and Milne et al. (18) examined ruminal contents and feces from a large number of herbivores for the presence of anaerobic fungi. Two species, impalas and blackbucks, were from the same subfamily as duikers, *Antilopinae*. Fungi were found in the ruminal contents of impalas but were not detected in blackbuck feces.

The high correlations between total numbers of bacteria and fungi and their concentrations in contents of ingesta were unexpected (Fig. 1). Although little information is available, it has been suggested that weight of contents is probably the major factor contributing to total microbial numbers. For example, a significant difference in protozoal concentrations between animals fed low- and high-roughage diets was found; however, when total protozoal numbers were calculated, the differences were no longer significant

(6). Obviously, concentrations and total numbers are not independent of each other; however, the low correlation observed between concentration and content weight indicates that for these data, concentration is the major factor determining total numbers.

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# ERRATA

## Bacterial and Fungal Numbers in Ruminal and Cecal Contents of the Blue Duiker (*Cephalophus monticola*)

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Volume 57, no. 2, p. 469, abstract, line 7: "high-forage diet" should read "high-concentrate diet"; "high-concentrate diet" should read "high-forage diet."

Lines 8 and 9: "high-forage and high-concentrate diets" should read "high-concentrate and high-forage diets."

Results, line 9: "high-forage diet" should read "high-concentrate diet."

Line 19: "high-concentrate diet" should read "high-forage diet."

Page 470, Table 3, column 1: "High forage" should read "High concentrate"; "High concentrate" should read "High forage."

Page 471, column 2, lines 38 and 39: "high-roughage diet" should read "high-concentrate diet"; "high-concentrate diet" should read "high-roughage diet."

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## Monohydroxylation of Phenol and 2,5-Dichlorophenol by Toluene Dioxygenase in *Pseudomonas putida* F1

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Volume 55, no. 10, p. 2650, Table 2, boxhead over columns 2 to 4: "μmol" should read "nmol."