COMPOSITION OF FRACTIONS OF THE RUMEN POPULATION OF DIFFERENT CELL SIZE

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The rumen microbial population has a structure that is related to cell size (Baker 1990). This size structure has been determined using a Coulter counter where cell size is expressed as the diameter of a sphere of the same volume as the cell, regardless of morphology. In this study centrifugal elutriation was used to separate the rumen population into fractions containing microorganisms of different cell size, in order to determine the microbial composition of each fraction.

Rumen contents from a mature wether fed a diet of oaten hay, lupins, and minerals (75:20:5) were collected 6 hours after feeding, and formaldehyde was added to a final concentration of 1% (v/v). The sample of rumen contents was strained and the filtrate was separated at 5°C into 5 fractions using an elutriation system and rotor (Beckman Instruments) under the following conditions; fraction 1, 1000 rpm and 50 ml/minute; fraction 2, 2700 rpm and 50 ml/minute; fraction 3, 5800 rpm and 15 ml/minute and fraction 4, 6000 rpm and 3 mL/minute. The material that was not retained in fraction 4 was designated fraction 5. The diluent used was 0.5% (v/v) formaldehyde in 0.9% (w/v) NaCl. Representative samples of each fraction were examined using bright field microscopy and microorganisms were identified using the schemes of Moir and Masson (1952) and Ogimoto and Imai (1981) in either unstained or brilliant-green stained preparations. The cell size of microorganisms in each fraction was measured using a Coulter counter and is expressed as the diameter of a sphere of the same volume.

There was very little cross-contamination between fractions and there were clear differences between the cell sizes and morphologies of organisms present in different fractions (Table 1). Where an organism was represented in 2 successive fractions the size of the organism was distinct between the 2 fractions, for example *Entodinium* in fracions 1 and 2. Zoospores were found only in fraction 3. The use of centrifugal elutriation to separate fractions of the rumen microbial population is a novel approach to the study of microbial ecology of the rumen in relation to the nutrition of the animal.

Table 1. The range of cell size (μm) in and composition of fractions of the rumen microbial population separated by centrifugal elutriation

Fraction	Size range	Type of particle (after Moir and Masson 1952; Ogimoto and Imai 1981)
1	30-100	*Diploplastron, Eodinium, Polyplastron, Entodinium, Dasytrichia, Isotrichia,* plant debris (<1% of particles).
2	10-30	*Entodinium, Dasytrichia,* large *Oscillospira,* plant debris (*ca.* 1% of particles).
3	3-10	Small *Oscillospira,* Quin's and Eadie's Ovals, zoospores, organisms 2, 5, 7, 18, plant debris (*ca.* 5% of particles)
4	1-3	Organisms 5, 17, 18, 21, coccobacilli, paired cocci
5	<1	Organisms 20, 21, 28, coccobacilli, rods with round ends, cocci, paired cocci.

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