CULTIVATION OF RUMEN ENTODINIOMORPHID PROTOZOA ON TROPICAL ANIMAL FEEDS

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The effect of a number of food materials, readily available for ruminants in Mexico on the growth in vitro of four species of rumen Entodiniomorphid protozoa was examined. Banana meal. dried banana leaves and rice polishings did not replace the usual food materials of rice starch, ground wheat and dried grass for the growth of *Entodinium caudatum, Epidinium ecaudatum caudatum, Ophryoscolex caudatus* or *Eudiplodinium maggii* and were not stimulatory when added as supplements to these substances. In contrast cassava meal supported the growth of *Entodinium caudatum* and *Epidinium ecaudatum caudatum* for over nine months at population densities comparable to those usually found. The possible significance of these findings to the feeding of ruminants is discussed.

Key words: Rumen protozoa, cultivation, banana, cassava, rice polishings

Rumen entodiniamorphid protozoa grow well under anaerobic conditions on reduced buffered salts media (principally potassium phosphate) provided that starch and/or ground dried grass is added each day to the medium (Coleman 1978). The starch used by the present author and others has usually been ground wheat although rice starch (Clarke 1963 Gutierrez & Davis 1962) and clover starch (Oxford 1958) have also been used. In the author's experience rice starch is only utilized by *Entodinium caudatum*, all the other species preferring whole ground wheat. The areas preparations used have included lawn mowings from English gardens (Coleman 1978), alfalfa (Gutierrez & Davis 1962; Rahman et al 1964), bluegrass or perennial rye grass (Clarke 1963; Jarvis & Hungate 1968), Italian winter rye grass (*Lolium italicum*) and winter grass (*Bromus catharticus*) (Hungate 1942) and prepared material from clover leaves (Oxford 1958). As no studies had been made on the cultivation of Entodiniomorphid protozoa on tropical feeds, an investigation was made of the ability of materials readily available in Yucatan and Tabasco, Mexico to support the growth of these protozoa.

Materials and Methods

Culture medium: The basal medium consisted of 26 ml caudatum-type salts medium, 0.3 ml of 2% (w/v) L-cysteine HCl (neutralised immediately before use) contained in a 50 ml centrifuge tube. Ninety five per cent (v/v) N2 + 5% (v/v) CO_2 , was bubbled vigorously through the medium for 2 min and the tube stoppered immediately with a rubber bung, *Entodinium caudatum*, unlike the other two species was routinely cultured in 100 ml centrifuge tubes containing three times the above quantities to which was added 42 μg chloramphenicol/ml. Caudatum type salts

medium contained (g/100 ml) : K_2HPO_4 , 0.63; KH_2PO_4, 0.50, NaCl,0.065; $CaCl_2$ (dried), 0.0045; $MgSO_4$,$7H_20$, 0.009; CH_3 OONa, 0.075.

Cultural conditions: Under standard conditions 0.2 ml 1.5% (w/v) aqueous suspension (or an equivalent amount of dry material) of the substrate under test was Jded daily and a small spatula end of ground dried grass (approximately 5 mg - Coleman 1978) twice a week to each culture tube which was then regassed with 100% CO_2. Once a week the contents of each tube were mixed, half poured into another tube and both made up to the original volume with fresh medium. All cultures were incubated at 39°C,

Source of protozoa: All protozoa were isolated from the rumens of Clun Forest sheep at Babraham, England, fed once daily on hay (800 g) and oats (100 g). *Entodinium caudatum* was isolated in 1959 by enrichment and fed daily thereafter on rice starch and coarsely ground dried grass. *Epidinium ecaudatum caudatum* was isolated by inoculation of single protozoa into medium in October 1977 and fed daily on ground wheat and coarsely-ground dried grass. *Eudiplodinium maggii* was isolated in the same way in December 1975 and fed daily on finely ground dried~grass. *Ophryoscolex caudatus* was isolated in July 1979 and grown on standard medium containing 10% (v/v) prepared freah rumen fluid (Coleman 1978), It was fed daily on ground wheat and coarselyground dried grass.

Tropical substrates: Rice polishings, banana meal and dried henequen pulp were kindly aupplied by Dr. R. Elliott of Escuela de Medicina Veterinaria y Zootecnia in Merida, Yucatan and cassava meal and dried banana leaves by the staff at Colegio Superio: de Agricultura Tropical at Cardenas, Tabasco.

Protozoal counts: These were alwaya made immediately before diluting a culture as described by Coleman (1978). Where the effect of a change in growth medium is reported, at least three weeks were allowed to elapse before the number of protozoa was estimated.

Results

Entodinium caudatum: Although *E caudatum* was normally grown in the presence of 10% (v/v) autoclaved rumen fruit, this was omitted from most of the present studies in order to make the growth conditions for all protozoa! species comparable. Banana meal supported a normal protozoal population density for only a few days after which time the numbers of protozoa declined steadily. One culture died after three weeks although another had 100/ml present after six weeks. Ground tried banana leaves and rice polishings would not replace the dried grass in the culture and did not increase survival time in the presence of the banana meal. In contrast cassava meal supported protozoal growth for at least nine months at a population density of 2900/ml compared with 4600/ml on rice starch. In the presence of 10% autoclaved rumen fluid the comparable densities were 2000 and 12,000/ml.

Epidinium ecaudatum caudatum: In the presence of banana meal and dried grass, *E. ecaudatum caudatum* grew normally for four weeks, but the population density then declined and all the protozoa died after nine weeks. On replacement of the dried grass by ground banana leaves, the cultures grew well for only one week and died after four weeks. Henequen pulp and rice polishings, which is potentially a

rich source of growth factors, were both inhibitory to protozoa! growth and when added in addition to ground wheat killed cultures in less than a week. As with *Entodinium caudatum* cassava meal supported cultures of *Epidinium ecaudatum caudatum* for over nine months. However the population density of 200/ml was less than that obtained with ground wheat. In the absence of the dried grass the corresponding densities were 66 and 160/ml compared with 650/ml if the ground wheat used was coated with β-sitosterol as described for rice starch by Onodera & Henderson (1980). This suggests that both cassava meal and wheat were deficient in sterol which is essential for protozoa! growth.

Eudiplodinium maggii: Replacement of the normal food material (ground dried grass) by ground dried banana leaves or henequen pulp resulted in death of the protozoa in 2 - 3 days. Although *E. maggii* will grow on ground wheat and dried grass, the cultures died in less than a week in the presence of banana meal, cassava meal and rice polishings whether or not they were also given dried grass.

Ophryoscoles caudatus: Attempts to grow this large starch engulfing protozoon on banana meal and dried grass instead of the normal food of ground wheat and dried grass resulted in a decline in the population density from 140/ml to 17/ml after 4 weeks and to less than 1/ml after 6 weeks. In the presence of the normal food, rice polishings were neither inhibitory nor stimulatory to growth.

Discussion

The failure of banana meal which is readily available in many tropical countries, to support growth of even the largest protozoa is probably due to the large size of its starch grains. Many of these, which are over 30 µm long, are therefore too large to be engulfed by the *Entodinium* spp which are the protozoa! species mainly responsible for sequestering starch in the rumen and rendering it unavailable for bacterial attack. The starch grains in cassava meal are intermediate in size between those of wheat and banana and are engulfable by *Entodinium caudatum* and the larger protozoa. These results therefore suggest that if a starch-rich supplement is to be fed to ruminants, cassava meal might be preferable to banana meal as being less likely to cause acidosis resulting from a rapid breakdown of starch by bacteria.

The finding that rice polishings do not stimulate the growth of protozoa supports those of Valdez et al (1977) who found that, although this material stimulates the growth of ruminants, it had no effect on the protozoa! biomass.

The failure of the protozoa to utilize the ground dried banana leaves is less easy to explain but as the history of this material is not known it could have been that the drying was done at too high a temperature. Heating of grass in an oven at 160°C is known to produce a preparation that is toxic to protozoa (Coleman 1978).

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