MURALYTIC ACTIVITIES OF FUNGAL AND BACTERIAL FRACTIONS OF DEFAUNATED RUMEN FLUID AGAINST INTACT OR NEUTRAL DETERGENT-TREATED ROUGHAGES

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Introduction

Anaerobic rumen fungi are now considered to be one of important elements which are involved in fibre digestion. As suggested by Bird and Leng (1984), their contribution would be unnegligible in defaunated ruminants in which activities of cellulolytic bacteria are reduced in addition to the absence of cellulolytic protozoa. However their quantitative contribution to fibre digestion is still unclear especially in defaunated ruminants. In this experiments, muralytic activities of fungal and bacterial fractions of defaunated rumen fluid were measured by *in vitro* cultures with rice straw (RS), timothy hay(TM), alfalfa hay(AF) or their respective neutral detergent residues (RSND, TMND, AFND).

Materials and Methods

Two adult wether sheep each fitted with rumen cannula were used as donors of rumen fluid. They were defaunated prior to the experiment. The sheep received 400 g timothy hay and 200 g commercial concentrate twice a day at 9:00 and 18:00. Water and mineral block were always accessible to the sheep. Rumen fluid were taken from two sheep just before morning feeding and combined. The fluid was then squeezed through four-layers of surgical gauze. Substrates (500 mg) were placed in 100-ml conical flasks to which was added 10 ml of strained rumen fluid and 40 ml anaerobic McDougall buffer. All the manipulations were done under the stream of CO_2. The systems were then closed by Bunsene valves and incubated for 48 h at 39°C with continual shaking. At the end of incubation, the flasks were immersed in iced water to halt the fermentations. Culture fluids were centrifuged at 1,500 x g for 20 min, pellets were succesively analyzed for DM, NDF, ADF, Klason lignin (ADL), and ash. Values of fibre components were corrected for their ash contents. Degradabilities of these components were estimated by the comparison with their initial values which were determined in unincubated cultures. Streptomycin sulphate and penicillin G potassium were added to the cultures in order to estimate fungal activity, cycloheximide was used for bacterial activity. Initial concentrations of these antibiotics per unit of strained rumen fluid were the same as Windham and Akin (1984). Anaerobic colony counts for fungi and bacteria were done respectively by the method of Joblin (1981) and RGCA roll rube method. Number of cellulolytic and xylanolytic organisms were estimated by most probable number method.

Results and Discussion

1.1–2.1 x 10^3 viable fungal zoospores/ml were detected in the rumen of sheep. Fungi were assigned as *Neocallimastix* spp. by their monocentric thalli and polyflagellated zoospores. Total viable bacteria were 3.3 x 10^9/ml. Numbers of cellulolytic and xylanolytic organisms were 1.9 x 10^8/ml and 1.5 x 10^9/ml respectively. Degradabilities of all the components (table 1) measured with bacteria alone (B) corresponded to at least 80% of those measured in combined cultures (BF, no antibiotics). Those measured with fungi alone (F) for NDF and ADF corresponded to approximately 50% of BF cultures with intact substrates, while they were reduced to less than 20% with treated substrates. Apparent depression in fungal activity against treated substrates suggests the necessity of neutral detergent soluble matters for fungal development and/or related enzyme production. Microscopic observations did not detect any apparent difference in the extent of fungal colonization between intact and treated substrates, possibly suggest that reduced fungal digestion is due to reduced enzyme production. ADL degradabilities in F cultures, corresponding at least 70% of those

TABLE 1. MURALYTIC ACTIVITIES OF FUNGAL AND BACTERIAL FRACTIONS OF DEFAUNATED RUMEN FLUID (% DEGRADED IN 48 H)

Substrate	BF	B	F	SEM
		NDF		
RS	48.2^{b}	37.5^{cd}	25.9^{e}	1.4
RSND	39.5^{cd}	33.9^{d}	5.2^{f}	
TM	56.6^{a}	51.3^{ab}	36.8^{de}	
TMND	48.3^{b}	39.8^{cd}	6.8^{f}	
AF	44.5^{bc}	43.8^{bc}	24.7^{e}	
AFND	39.5^{cd}	38.2^{cd}	7.7^{f}	
		ADF		
RS	47.8^{b}	38.5^{d}	27.5^{f}	1.4
RSND	37.2^{d}	31.3^{e}	5.3^{h}	
TM	56.2^{a}	49.5^{b}	36.1^{de}	
TMND	44.8^{bcd}	35.9^{de}	8.7^{gh}	
AF	38.7^{d}	39.4^{cd}	11.8^{g}	
AFND	39.1^{cd}	37.7^{d}	7.2^{h}	
		ADL		
RS	29.2^{ab}	35.3^{b}	21.3^{bc}	1.1
RSND	25.4^{bc}	23.2^{bc}	17.9^{bc}	
TM	29.2^{b}	30.9^{ab}	20.2^{bc}	
TMND	21.5^{bc}	19.2^{bc}	16.5^{bc}	
AF	11.8^{c}	17.6^{ac}	14.0^{bc}	
AFND	12.3^{c}	11.8^{c}	4.2^{d}	

BF, combined culture; B, bacteria alone; F, fungi alone.

Values are means of six determinations. Values with different superscript within rows within a component differ ($p < 0.05$).

in BF cultures, were high. These results suggest the capacity of fungi for lignin degradation. Sum of degradabilities in F and B cultures surpassed those in BF cultures in most cases, this suggests the presence of antagonism between these two organisms. The extent of fungal colonization was, indeed, suppressed by the presence of bacteria. (Key Words: Rumen Fungi, Fibre Digestion, Interaction with Bacteria)

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