APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1985, p. 1329–1331
0099-2240/85/051329-03$02.00/0

Vol. 49, No. 5

Factors Stimulating Migration of Holotrich Protozoa into the Rumen

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Received 26 November 1984/Accepted 18 February 1985

The effects of feeding and various reticular infusions on ruminal holotrich concentrations were studied in an attempt to identify possible factors stimulating their migration into the rumen. It was concluded that glucose entering the reticulo-rumen shortly after feeding could stimulate migration of holotrich protozoa.

In frequently fed ruminants, protozoal nitrogen has been estimated to account for over 20% of the total nonammonia nitrogen reaching the duodenum (9). Although holotrichs are usually a small proportion of the total protozoal population, they make a significant contribution to total protozoal nitrogen and the nutrition of the host because of their relatively large size (1). For example, *Dasytricha* sp. averaged 5.5% of total counts but were estimated to account for 16.6% of total protozoal dry matter in sheep fed hourly (2). This effect would also apply to the larger but less numerous *Isotricha* spp.

Holotrich protozoa have been shown to sequester on the wall of the reticulum and migrate into the rumen for only a few hours after feeding (1). Chemical stimuli originating from the diet are thought to be involved in causing this migration; however, the physical act of feed ingestion per se has also been implicated. In addition, protozoal numbers have been observed to increase before feeding (3), the same time at which rumination activity would be expected to peak (7). Copious salivation occurs during both feed ingestion and rumination; therefore, saliva or a salivary component could be involved in stimulating holotrich migration into the rumen.

Chemotaxis of holotrichs to major soluble carbohydrates in plants (e.g., sucrose, glucose, and fructose) has been shown in vitro (8). Attempts to demonstrate this in vivo by administration of either 0.7 kg of corn starch or 0.2 kg of glucose through a rumen cannula were not successful (1). Our objective was to investigate the effects of feeding and various reticular infusions on ruminal holotrich concentration in an attempt to identify factors stimulating their migration into the rumen from a sequestered position, presumably on the wall of the reticulum (1). Based on the work of Clarke et al. (2), which indicated a large variation between animals in numbers and mass of ruminal ciliates, measures of variation and appropriate statistical techniques to assess treatment differences were included in our study.

Two 590-kg rumen-cannulated Holstein steers were fed a maintenance ration consisting of 1.5 kg of coarsely chopped wheat straw and 2 kg of a concentrate mix twice daily (0930 and 1430 h). The concentrate mix contained 84.25% ground shelled corn, 13% soybean meal, 1.5% dicalcium phosphate, 1.2% trace mineralized salt, and 0.05% vitamin A and D supplement.

Composite samples of 100 ml of fluid from the ventral rumen, removed by a suction strainer inserted through the rumen cannula, and 100 ml of fluid expressed from material in the dorsal rumen were taken hourly from each steer. Samples were filtered through one layer of cheesecloth. One volume of sample was mixed with 2 volumes of fixative-stain (5). Subsamples were further diluted with 50% glycerol–fixative-stain (1:20) and counted in duplicate. Protozoa concentrations were estimated by counting 20 microscopic fields in a cell (5 by 2 by 0.1 cm) at a magnification of ×150.

In a second experiment, 1.5 kg of chopped straw was placed into the rumen of a steer via cannula after sampling at 0900 h. Ruminal samples were taken hourly until 1300, after which 2 kg of concentrate mix was placed into the rumen and hourly samples were withdrawn until 1700 h. Samples were prepared and analyzed as described above. The other steer was fed and sampled in the same manner except that it received concentrate mix followed by chopped straw. Both steers were fed the remainder of their ration after 1700 h. The protocol was repeated 48 h later with the treatment order in each steer reversed.

In a third experiment, reticular infusions of 3 liters of distilled water, artificial saliva (6), 0.76 M glucose (equal to the estimated soluble carbohydrate content of the ration), corn starch slurry equivalent to glucose (368.2 g), or 0.46 M NaCl (isoosmotic to 0.76 M glucose) at 0.1 liters/min were assigned to each steer, once, in a random order with 48 h between infusions. Solutions were pumped through a polyethylene tube (inside diameter, 9.5 mm) which was placed through the rumen cannula and weighted to keep it in the reticulum. Rumen contents were sampled before the 30-min infusion and hourly for 5 h. Samples were prepared and analyzed as described above. Infusions were started after sampling at 0900 h; however, feeding was delayed on these days until 1500 h, at which time the steer was fed the total ration. Statistical analysis of reticular infusion data was performed by using a general linear model procedure (SAS Institute Inc., Cary, N.C.) except that a "conservative" F procedure was used for testing time and treatment by time interaction (4).

The holotrich concentration in the rumen over 24 h is shown in Fig. 1. Ruminal holotrich concentration increased about 10-fold in the 2 h after each feeding but returned to the prefeeding level in 5 to 6 h.

A threefold increase in ruminal holotrich concentration was caused by placing 1.5 kg of chopped straw or 2 kg of concentrate mix directly into the rumen after 0900 h (Fig. 2). Administration of concentrate mix after 1300 h also caused a threefold increase in ruminal holotrich concentration; how-

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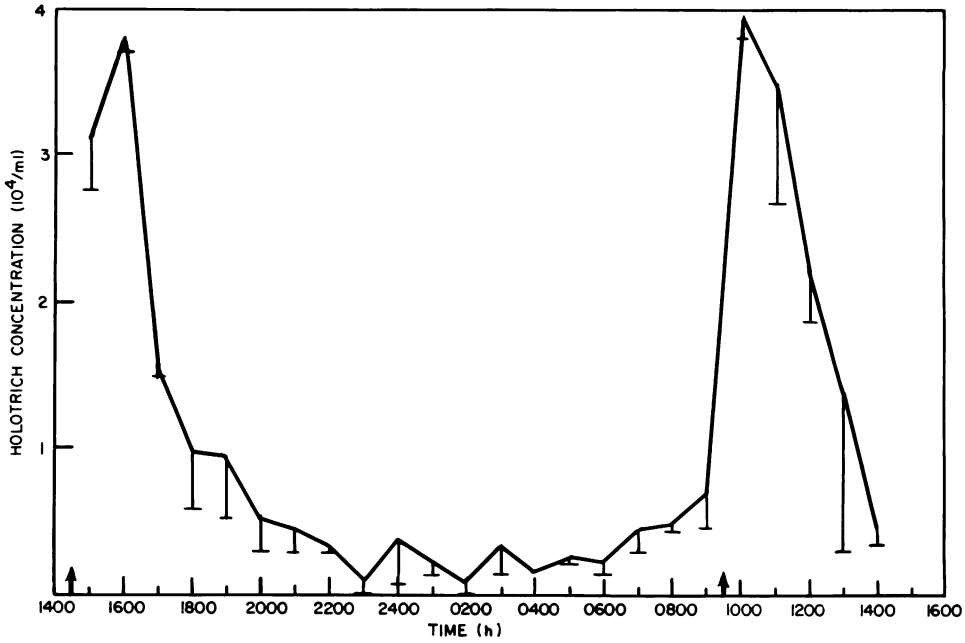

FIG. 1. Mean ruminal holotrich concentration in two steers throughout the day. Arrows indicate when steers were fed 1.5 kg of coarsely chopped wheat straw and 2 kg of concentrate mix. Vertical bars denote the standard error of the mean at each sampling time (n = 2).

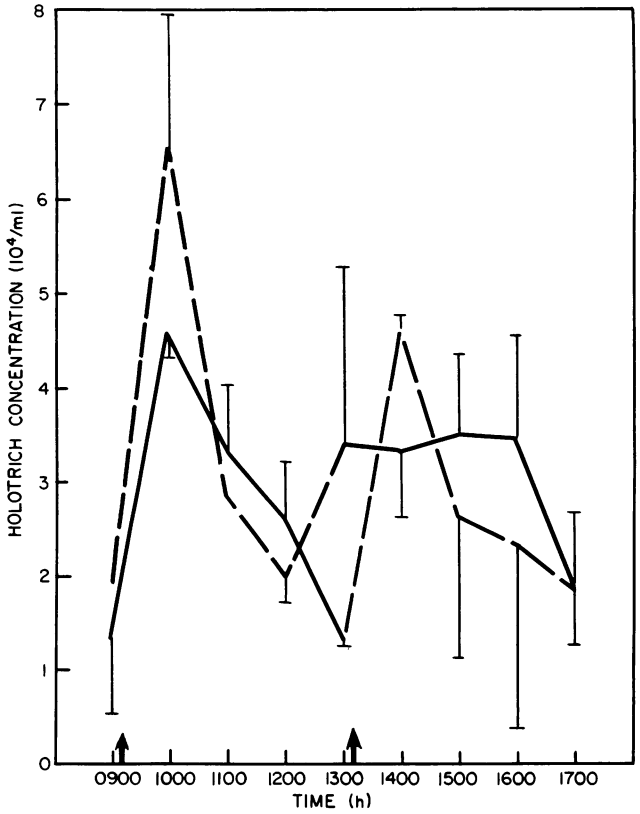

FIG. 2. Mean ruminal holotrich concentration in two steers as affected by placement of 1.5 kg of chopped straw (——) or 2 kg of concentrate mix (– – –), alternately, directly into the rumen after sampling at 0900 or 1300 h (arrows). Vertical bars indicate the standard error of the mean at each sampling time (n = 2).

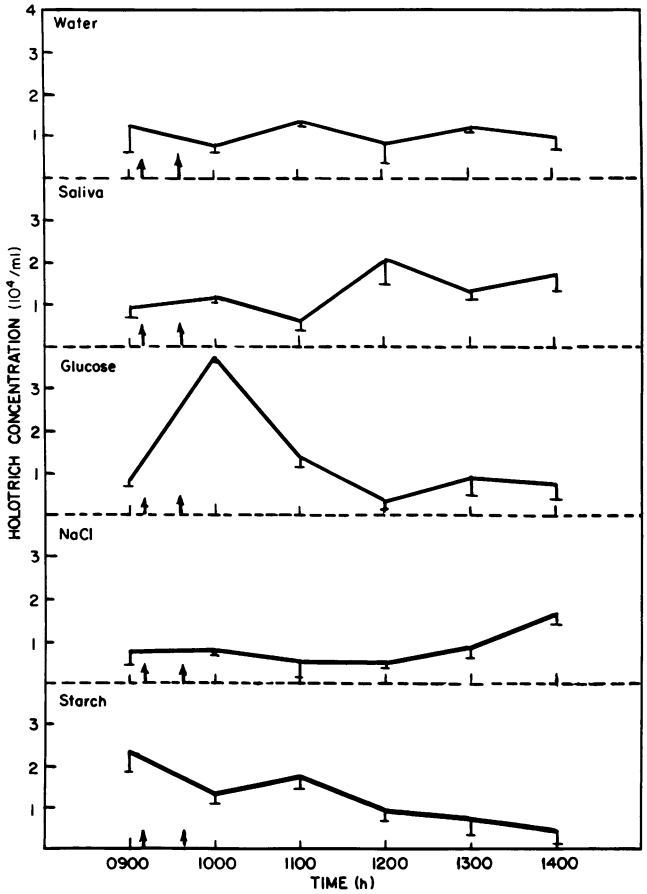

FIG. 3. Mean ruminal holotrich concentration in two steers as affected by reticular infusion of 3 liters of distilled water, artificial saliva, 0.76 M glucose, 0.46 NaCl, or corn starch slurry (368.2 g). Arrows indicate the infusion interval and vertical bars indicate the standard error of the mean at each sampling time (n = 2).

ever, a smaller and more variable response was found when chopped straw was added to the rumen in the afternoon.

Effects of reticular infusions on ruminal holotrich concentration are shown in Fig. 3. Treatment by time interaction was significant ($P < 0.025$, "conservative" F test) and indicated that reticular infusion of glucose was accompanied by holotrich migration into the rumen which helped increase their concentration fivefold.

In agreement with Abe et al. (1) but not with Dehority and Mattos (3), no prefeeding increase in ruminal holotrich concentration was noted (Fig. 1). Rumination of wheat straw, which is similar to rice straw (1) but perhaps different from Rhodes grass hay (3), would not be expected to release significant quantities of soluble carbohydrate. A prefeeding increase in ruminal holotrich concentration (3) may depend on forage quality and time of feeding in relation to peak rumination activity.

The act of feed ingestion is probably less important than previously suggested (1) because even a single ration component placed directly into the rumen elicited substantial and transient increases in holotrich concentration (Fig. 2). Peak concentrations were similar to those found when both ration components were fed together (cf. Fig. 1 and 2).

Holotrich migration into the rumen was stimulated by reticular infusion of glucose (Fig. 3) but not other solutions. The return to baseline concentration within 2 h after reticular infusion of glucose may be explained by the normally rapid fermentation of soluble carbohydrates in the reticulo-rumen. This might also explain why Abe et al. (1) were unable to stimulate holotrich migration by placing 0.2 kg of glucose into the rumen. Rapid ruminal fermentation and dilution of this glucose by ruminal contents might have caused little change in glucose concentration in the reticulum where holotrich protozoa were presumably sequestered. It was concluded that glucose in the reticulo-rumen shortly after feeding could stimulate holotrich migration.

This work was supported by Illinois Agricultural Experiment Station, under Hatch Act grant 35-0360, and the Undergraduate Research/Scholarship Program, College of Agriculture, University of Illinois, Urbana.

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