

Diurnal Changes in Concentration of Rumen Ciliates and in Occurrence of Dividing Forms in Water Buffalo (*Bubalus bubalus*) Fed Once Daily

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When buffalo were fed once daily, significant diurnal variations in concentration of rumen ciliates and occurrence of dividing protozoa were found. Differences in proportions of dividing *Entodinium*- and *Diplodinium*-type ciliates were also observed. Results obtained suggest that the range of diurnal fluctuations in rumen protozoa concentration may be related to the percentage of dividing cells in populations of these organisms.

Diurnal fluctuations in concentration of rumen ciliates in animals fed once daily have been described by several authors (1, 3-7), but variations in dividing processes in populations of these organisms were examined only sporadically (6, 7). In the present paper the relationship between changes in concentration of ciliates and numbers of dividing cells of these protozoa in the rumen of water buffalo were estimated.

MATERIALS AND METHODS

Once daily, three adult water buffalo, with permanent rumen fistulas, were fed hay only (hay diet) or mixed food containing 13.3% (standard diet) or 26.9% (enriched diet) concentrates. A detailed description of the experiment and characteristics of daily rations have been described in a previous paper (2).

Samples of rumen contents for protozoal counts were taken from the rumen of each animal just before feeding and then every 2 h. They were fixed in formalin and counted using a light microscope.

RESULTS

Diurnal changes in concentration of *Entodinium*, as well as in the number and proportion of dividing organisms from this group, are presented in Fig. 1. The maximal concentration of these ciliates and minimal percentage of dividing forms were found just before giving the food. Feeding was associated with a drop in population density during the first 10 to 14 h and a decrease in the number of dividing protozoa during the first 2 to 4 h. On the other hand, a gradual increase in percentage of dividing cells during the first 10 to 12 h was found. In the last 10 to 14 h before the next feeding an increase in protozoal concentration and decrease

in proportions of dividing ciliates were observed, whereas the number of organisms undergoing division remained relatively stable ($P > 0.05$).

The diurnal variations of ciliates from the genus *Diplodinium* are presented in Fig. 2. The concentration of these organisms reached the maximum just before feeding and the minimum at 2 or 10 h after feeding. Dividing ciliates were at a relatively stable level during the whole 24-h period ($P > 0.05$) and formed a significantly larger part of the whole *Diplodinium* group than dividing *Entodinia* ($P < 0.01$). The greatest differences were found immediately before giving the food (2.3 to 4.4% of dividing *Entodinium*-type ciliates as compared to 7.1 to 13.6% of dividing protozoa from the genus *Diplodinium*). In every case the maximal percentage of organisms undergoing division was found at 8 to 12 h postfeeding. There were no significant fluctuations in the proportion of dividing *Diplodinia* observed in buffalo fed enriched diet ($P > 0.05$).

The diurnal fluctuations in the *Holotricha* concentration are presented in Fig. 3. It reached its maximum at the same time as *Entodinium* and *Diplodinium*. During the first 12 to 18 h postfeeding, the number of these organisms decreased by about 80 to 86% and increased five to six times during the last 6 to 8 h before feeding. No irregularities were found in occurrence of dividing ciliates from the subclass *Holotricha*, since they were observed rather sporadically. However, during the rapid increase in *Holotricha* concentration, numerous *Dasytricha* were observed and were much smaller than others, more oval, and distinctly lighter in color. They were probably immediately after division.

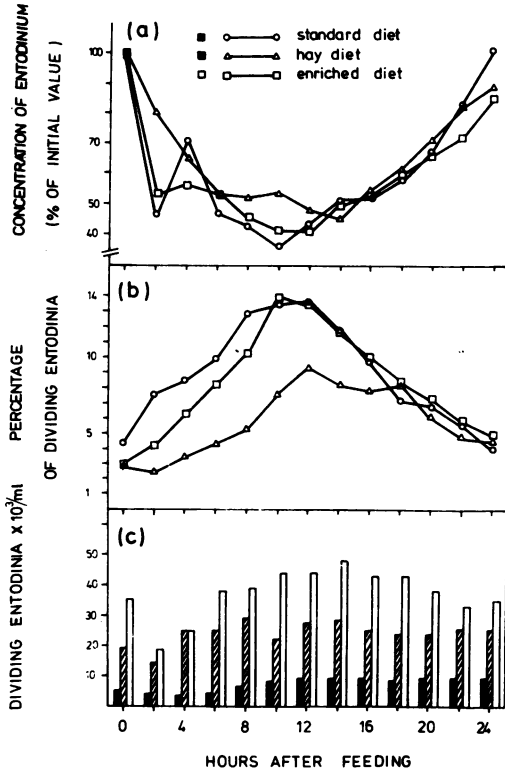


FIG. 1. Diurnal variations in concentration of *Entodinium* (a), percentage of dividing cells (b), and number of dividing cells (c) in buffalo fed three different diets.

DISCUSSION

In a previous paper I showed that changes of diet caused distinctive changes in protozoal concentration in the rumen of water buffalo (2). Results presented here, however, suggest that diet has only a small influence on the pattern of diurnal variations in ciliate concentration. That agrees with the previous observations of Warner (7) concerning diurnal fluctuations in ciliate concentration in the sheep rumen.

In all groups of protozoa the highest concentration occurred immediately before feeding (Fig. 1-3). After feeding, a drop in population density was observed, followed by an increase. The decrease in ciliate concentration was probably caused by the dilution of rumen contents by the food, water, saliva, and passage of digesta from the rumen. In the case of holotrichs, it was noted that the decrease in concentration was caused by bursting of cells of these organisms, due to abnormal synthesis of the storage carbohydrates (1); but in the present experiment burst *Holotricha* cells were observed only occasionally. Thus, during the

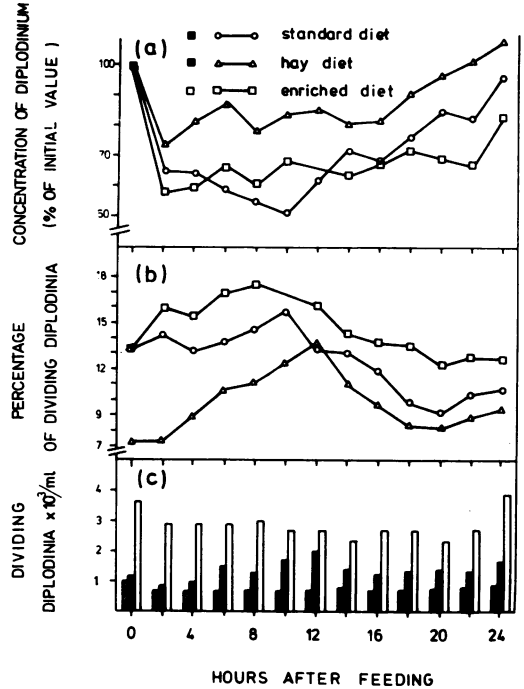


FIG. 2. Diurnal variations in concentration of *Diploplodinium* (a), percentage of dividing cells (b), and number of dividing cells (c) in buffalo fed three different diets.

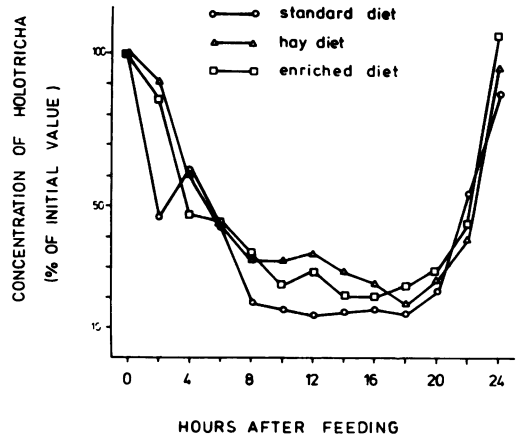


FIG. 3. Diurnal variations in concentration of *Holotricha* in buffalo fed three different diets.

decrease in their concentration, it is more probable that the growth rate of these organisms was smaller than the dilution rate of rumen contents.

The increase in ciliate density might be caused both by intensification of divisional processes and decrease in the dilution rate of rumen contents. There are some data (8) sug-

gesting that passage of protozoa from the rumen is slower than the outflow of rumen liquid, which phenomenon might be responsible for the increase in the protozoal concentration in the rumen. However, this increase in density of protozoa might occur when the volume of rumen contents has been decreased through outflow. However, the data of Wright and Grainger (9) and our unpublished observations indicate that in animals fed once daily the rumen content volume decreased only in the first 12 h after feeding. In the present experiment *Diplodinium* increased during this period. On the other hand, however, in this period the percentage of dividing cells in the *Diplodinium* group was significantly higher than in *Entodinium* (Fig. 1 and 2), and dividing holotrichs were observed only sporadically. Thus, the rate of multiplication of protozoa and its relation to the dilution rate of the rumen contents should be studied as the main factors causing diurnal changes in protozoal concentration.

Results obtained (Fig. 1) suggest that divisional processes among *Entodinium*-type ciliates increased during the 2- to 14-h postfeeding period and had a relatively stable rate during the last period. From data of Wright and Grainger (9) and from my investigations (unpublished data) it is evident that the dilution rate of rumen contents during the 12- to 24-h postfeeding period in sheep fed once daily is several times smaller than that during the first 12 h. If these relationships in water buffalo are the same (these investigations, unfortunately, have not been done), the gradual increase in *Entodinium* concentration would be possible mainly in the second half of the postfeeding period. A higher proportion of dividing cells in the *Diplodinium* group as compared with that in *Entodinium* (see Fig. 1 and 2) would cause an earlier increase in density of these ciliates.

The increase in *Holotricha* concentration in the last 6 to 8 h before feeding cannot be explained by the decrease in dilution rate in this period. My investigations (unpublished data) indicate that this decrease is not great enough to explain the increase in *Holotricha*. Moreover, the dilution rate was actually increased during the last 4-h postfeeding period. Therefore, the increase in number of holotrichs has to be mainly the result of a rapid increase in the multiplication rate of these organisms. This suggestion is supported by the occurrence of small *Dasytricha* cells assumed to represent the growth stage immediately after division.

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