Periodic Changes in the Concentrations of Micro-organisms in the Rumen of a Sheep Fed a Limited Ration every Three Hours

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SUMMARY

A sheep was given small amounts of feed at 3-hr intervals and the concentrations of several groups of micro-organisms in the rumen were measured at intervals over 28 hr. A 3-hr rhythm of concentration change was seen for most micro-organisms. Superimposed on this was a gradual decline in concentration of several groups of micro-organisms, presumably due to removal of nutrients by repeated sampling from the rumen.

INTRODUCTION

In the preceding paper (Warner, 1966) the pattern of changes in concentration of the micro-organisms in the rumen of sheep fed a limited ration once daily was shown to depend primarily on the nature of the micro-organisms and the time of feeding. No evidence for any other diurnal rhythm was found. The present paper reports a study of the changes in concentration of the rumen microbes in a sheep fed at 3-hr intervals. The aim was to determine whether nearly constant conditions in the rumen could be achieved. The frequently repeated, regular sequence of observations also allowed a study of the effects of repeated sampling on the micro-organisms.

METHODS

An English Leicester-Merino crossbred ewe S074 was given 89 ± 6 g. of the diet R9 (equal parts of lucerne and wheaten chaffs) every 3 hr by means of an automatic dispenser. The animal nearly always consumed this feed within 5 min. Water was available at all times. Before the start of the experiment, the animal had spent 6 weeks on this routine, and before that had been fed 700 g. diet R9 once daily.

On the day of the experiment, samples (about 50 ml.) of rumen contents were removed through a fistula; care was taken to collect the material from several sites within the rumen. The pH value was determined without delay. The remainder of the specimen was strained and sampled for microbiological examination by the standard technique of Warner (1962*a*); nomenclature of the organisms followed Warner (1966). A further portion of the strained rumen liquor was diluted with twice its volume of 0.1 N-HCl, allowed to stand at least 10 min., and filtered. Estimations of ammonia and volatile fatty acids were made on the filtrate by the

methods of Warner (1964) and Warner & Stacy (1965), respectively. Rumen samples were removed at intervals throughout 28 hr, at one of 3 times, 0.5, 1.5, 2.5 hr after the time of feeding.

RESULTS

When the results concerning a particular micro-organism were considered within three groups, namely those for 0.5, 1.5 and 2.5 hr after feeding, respectively, it was found that for some species the concentrations showed overall decreases with increasing time measured from the beginning of the experiment. For micro-organisms for which the data suggested the possibility of such a decrease, instead of an ordinary analysis of variance to compare results of the three groups of observations, a simple linear regression analysis against time from the beginning of the experiment was carried out for each group. The three corresponding regression coefficients and

Fig. 1. Concentration of entodinia, dasytrichs and selenomonads in the rumen of a sheep fed at 3-hr intervals. The lines connect points plotted for the calculated mean values, allowing for the regression due to time (see text). The vertical lines indicate the time of feeding.

their standard errors were computed and then, assuming that the three lines were in fact parallel, a common gradient and its standard error was then found by pooling regression results from the groups. By removal of the appropriate regression sums of squares, an analysis of covariance was then made to discover whether there was any difference between the three groups not due to the time after the beginning of the experiment.

Where no overall decline as described above was suspected, the means of values found for each time after feeding were entered in Table 1. Where such a decline

Table 1. *Micro-organisms and metabolites in the rumen of a sheep fed at 3-hr intervals*

	Mean, calculated to zero time, $\pm$ S.D. at indicated times after feeding			Significance* of difference* (F test)	Regression gradient per hr
	0.5 hr	1.5 hr	2.5 hr		
pH value	6.39 $\pm$ 0.08	6.45 $\pm$ 0.03	6.53 $\pm$ 0.03	***	—
Volatile fatty acids (m-equiv./l.)	95 $\pm$ 6	93 $\pm$ 8	84 $\pm$ 12	**	—
Ammonia (m-equiv./l.)	14.1 $\pm$ 1.2	12.7 $\pm$ 1.2	11.9 $\pm$ 1.0	**	—
Entodinia (concn. $\times 10^{-3}$ /ml.)	208 $\pm$ 16	205 $\pm$ 17	196 $\pm$ 14	n.s.	—
Proportion of entodinia dividing	0.032 $\pm$ 0.005	0.023 $\pm$ 0.003	0.021 $\pm$ 0.004	***	-1.4 $\pm$ 0.4
Diploplastrons (concn. $\times 10^{-3}$ /ml.)	15.7 $\pm$ 2.2	15.7 $\pm$ 2.2	14.6 $\pm$ 2.6	n.s.	-0.005 $\pm$ 0.001
Proportion of diploplastrons dividing	0.034 $\pm$ 0.006	0.030 $\pm$ 0.006	0.035 $\pm$ 0.011	n.s.	-0.03 $\pm$ 0.06
Dasytrichs (concn. $\times 10^{-3}$ /ml.)	46 $\pm$ 1	32 $\pm$ 6	28 $\pm$ 5	***	0.0001 $\pm$ 0.0002
Proportion of dasytrichs dividing	0.005 $\pm$ 0.001	0.005 $\pm$ 0.003	0.011 $\pm$ 0.003	*	-0.3 $\pm$ 0.1
Proportion of dasytrichs conjugating	0.001	0.002	0.002	n.s.	—
Isotrichs (concn. $\times 10^{-3}$ /ml.)	15 $\pm$ 4	9 $\pm$ 2	9 $\pm$ 3	**	-0.2 $\pm$ 0.1
Proportion of isotrichs dividing	0.003 $\pm$ 0.002	0.005 $\pm$ 0.003	0.008 $\pm$ 0.003	*	—
Polymastigates (concn. $\times 10^{-3}$ /ml.)	35 $\pm$ 6	18 $\pm$ 5	10 $\pm$ 6	***	—
Oscillospirae (concn. $\times 10^{-3}$ /ml.)	115 $\pm$ 9	94 $\pm$ 7	91 $\pm$ 6	***	-0.8 $\pm$ 0.2
Selenomonads (concn. $\times 10^{-6}$ /ml.)	173 $\pm$ 11	161 $\pm$ 7	151 $\pm$ 7	***	-0.3 $\pm$ 0.2
Total bacteria (concn. $\times 10^{-8}$ /ml.)	177 $\pm$ 7	166 $\pm$ 10	167 $\pm$ 11	n.s.	0.3 $\pm$ 0.2

* When a figure is given for the gradient in the next column, significance was calculated as described in the text, otherwise a normal analysis of variance was used. n.s. = not significantly different; *, **, *** = significantly different at levels of probability $P < 0.05$, 0.01, 0.001 respectively.

seemed possible, the experimental values were corrected to zero time by using the calculated regression coefficient and the means then computed. In Fig. 1 the actual, experimental values are plotted, together with a line joining points calculated from the means described above and the regression coefficient. It can be seen that, even when fed as frequently as at 8-hr intervals, considerable rhythmical fluctuations in concentration of both micro-organisms and metabolites occurred due to the feeding regime, though these were much less marked than when the animal was fed only once daily (Warner, 1966). No evidence was seen in Fig. 1 or in the other unpublished curves for any other diurnal rhythms.

DISCUSSION

Immediately following feeding there was an increase in metabolic activity, shown by the increase in ammonia and volatile fatty acid concentration and the decrease in pH value. All micro-organisms showed some degree of increase in concentration immediately after feeding, presumably due to the increase in nutrients, but the relative magnitude of this increase and the subsequent pattern of decline were characteristic of the micro-organism (Fig. 1).

The entodinia (Table 1) showed a small but statistically non-significant increase in concentration after feeding, and a considerable and highly significant increase in the proportion of dividing organisms. It is presumed that the reason why the considerably increased growth rate caused little increase in concentration was that the dilution rate also increased due to the increased flow of saliva accompanying feeding (Warner, 1966). The diploplastrons also showed a small but non-significant increase in concentration after feeding but, perhaps owing to greater variability, no significant difference in the proportion of dividing organisms was seen. The post-feeding increase in total bacterial concentration was also non-significant.

The concentration of selenomonads declined steadily, that of the oscillospirae at a decreasing rate, following the highly significant post-feeding rise. As in the sheep fed once daily (Warner, 1966) the polymastigates showed the greatest increase in concentration of all micro-organisms.

The holotrich ciliates showed minimal concentration and maximal proportion of dividing organisms immediately before feeding, and vice versa immediately after. In view of the previous work (Warner, 1966) one may speculate whether the stimulus to division was the immediately preceding feed or one considerably earlier. A few conjugating dasytrichs were seen in many specimens.

Over the 28 hr of the experiment there was a slow decline in the concentration of entodinia, holotrichs, oscillospirae and selenomonads. The proportion of dividing entodinia also declined, indicating inhibition of reproduction. During this 28 hr some 1000 ml. rumen contents were removed in sampling; this material would contain feed particles in various stages of degradation, soluble feed constituents, metabolic products and microbial cells; that is, nutrients for many of the rumen micro-organisms.

No overall decline in concentration was seen with the diploplastrons, but counting accuracy was comparatively low. The polymastigates maintained their numbers; if the suggestion of Warner (1966) is correct, then these organisms spend a considerable time sequestered close to the rumen wall, presumably for nutritional reasons,

so that the sampling procedure would remove proportionately less of these organisms, and the nutrition of the remainder would be little affected. The total bacterial count also did not decline, indeed there may have been a small increase. It is probable that many of the organisms counted here were normally 'fixed' species, in transit between one feed particle and another; since the larger feed particles were not removed at sampling, a considerable reservoir of organisms and their substrates would remain.

The holotrichs decreased in concentration more than the ophryoscolecids. A similar finding was made in starving cattle by Meiske, Salsbury, Hoefer & Lueckel (1958). This marked decrease in protozoa may have allowed a compensatory increase in the bacteria; defaunated animals commonly have high bacterial counts (Eadie, Hobson & Mann, 1959; Bryant & Small, 1960; Eadie & Hobson, 1962; Walker & Hope, 1964; see also Warner, 1962*b*).

Purser & Moir (1959) collected from sheep 8 specimens of rumen liquor of at least 100 ml. each, on each of 3 consecutive days. They found that the ophryoscolecid protozoa decreased considerably in mean daily concentration over the 3 days. This was associated with a decrease in minimal pH value. In the work reported here there was no change in pH value, while in the starvation experiments of Meiske *et al.* (1958) the pH value increased. While the causes are still uncertain, this work emphasizes that removal of a significant amount of rumen contents affects the remaining micro-organisms and their metabolism.

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