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Rôle of the rumen ciliate protozoa *Polyplastron multivesiculatum*, *Entodinium* sp. and *Isotricha prostoma* in the digestion of a mixed diet in sheep

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Summary. Six rumen-fistulated and defaunated wethers were fed twice daily a pelleted diet of barley and dehydrated lucern at a rate of 50 g dry matter/kg body weight $^{0.75}$. Two months later, the sheep were divided into three equal groups. Each group was then inoculated in the rumen with one of the following three genera of protozoa : *Polyplastron multivesiculatum* (*P*), *Entodinium sp.* (*E*), or *Isotricha prostoma* (*I*). After two months, each group was subdivided and inoculated with an additional genus to obtain two sheep for each *P* + *E*, *P* + *I* and *E* + *I* combination. After an additional two months, two sheep were inoculated with a mixture of several genera to restore them to a conventional state. At the end of each of the four two-month periods, the total digestibility of organic matter (OM), acid detergent fiber (ADF), nitrogen (N) and fermentation in the rumen were measured.

The *P* + *E* genera combination could not be obtained because *E* disappeared when the sheep were inoculated with the *P* species. However, these two genera can coexist in a rumen containing at least four different genera of protozoa.

OM and ADF digestibilities were improved mainly by inoculating the *P* genus and, to a lesser extent, the *E* genus, but the effect of *I* was negative. N digestibility tended to be higher after ciliate inoculation, compared to the defaunated state. Furthermore, the pH in inoculated sheep was generally lower, and volatile fatty acid (VFA) and ammonia levels were higher.

The composition of the VFA mixture was strongly influenced by inoculation with the *E* genus : the propionic acid proportion increased at the expense of the acetic and butyric acid proportions. This result was confirmed by the lower CH_4/CO_2 ratio observed in the rumen gas composition in sheep inoculated with the *E* genus. With *P* alone or with other ciliates, the butyric acid proportion increased. The rumen lactic acid concentration increased after inoculation with the *I* genus. In the conventional animals, fermentation patterns were similar to those observed in the *P*-inoculated animals but the differences increased and became significant.

Of the three ciliates studied, the *P* genus had the greatest effect on the digestive parameters which were similar to those found in conventional sheep. This effect could be explained by the ability of the *P* genus to hydrolyse and ferment most carbohydrates and by its predatory action on bacteria and protozoa.

Introduction.

Digestion in the rumen is mainly carried out by bacteria and ciliate protozoa. Although the role of bacteria in digestion is well-known, the effect of ciliates has long been unknown or considered to be negligible (Pounden and Hibbs, 1950 ; Eadie and Gill, 1971 ; Williams and Dinusson, 1973). Over the past twenty years, many studies comparing conventional (naturally faunated) animals with protozoa-free (ciliate-free) animals have been carried out. However, no definite conclusions have been drawn on ciliates because of the contradictory results obtained on growing animals (Bryant and Small, 1960 ; Eadie, 1962 ; Abou Akkada and El Shazly, 1964 ; Christiansen, Kawashima and Burroughs, 1965 ; Borhami *et al.*, 1967 ; Chalmers *et al.*, 1968) and on feed digestion (Abou Akkada and El Shazly, 1964, 1965 ; Barringer, Trenkle and Burroughs, 1966 ; Klopfenstein, Purser and Tyznik, 1966 ; Luther, Trenkle and Burroughs, 1966 ; Kurihara *et al.*, 1968 ; Males and Purser, 1970 ; Itabashi and Kandatsu, 1975 ; Jouany, 1975). Ciliates have shown wide differences in their effects *in vitro* on carbohydrate metabolism (Abou Akkada, 1965 ; Hungate, 1966 ; Church, 1975 ; Clarke, 1977). Merely a limited number of studies on the specific role of ciliates in the digestive process have been carried out *in vivo* (Christiansen *et al.*, 1965 ; Williams and Dinusson, 1973). Jouany (1978) only recently did a systematic study on the role of ciliates in carbohydrate digestion in the rumen.

Previous studies on bacteria-protozoa interrelationships have shown that bacterial concentration is lower when rumen ciliates are present (Bryant and Small, 1960 ; Eadie and Hobson, 1962 ; Kurihara *et al.*, 1968 ; Eadie and Gill, 1971), but the effect of inoculating each particular ciliate genus separately on the bacterial population has rarely been studied *in vivo*.

In the present experiment, we measured the *in vivo* effects on the digestion of *Polyplastron multivesiculatum* (*P*), *Entodinium sp.* (*E*), and *Isotricha prostoma* (*I*) inoculated into the defaunated rumen of sheep. The sheep with these genera, inoculated as single or combined species, were compared with totally faunated (TF) sheep. We also attempted to determine the effect of inoculating each of these three genera on the composition of the rumen bacterial population. At the same time, we studied the dynamics of the ciliate populations in all the inoculated combinations (Senaud *et al.*, 1980 ; Grain *et al.*, 1980).

Material and methods.

Experimental procedure.

Diet. — The diet was composed of barley (49 p. 100), dehydrated lucerne (40 p. 100), wheat straw (9 p. 100), NaCl (0.45 p. 100), $CaHPO_4$ (0.5 p. 100) and a vitamin-mineral mixture (1.05 p. 100). Dry matter accounted for 91.0 p. 100 of the fresh weight. The pelleted diet (diameter : 1 cm) contained (dry-matter basis) : crude protein (N $\times$ 6.25), 13.6 p. 100 ; starch, 27.2 p. 100 ; acid detergent fiber (ADF), 24.3 p. 100 ; demineralized ADF, 20.6 p. 100 ; neutral detergent fiber (NDF), 39.5 p. 100 and organic matter, 92.4 p. 100. This diet was used to obtain high numbers of rumen ciliates.

Animals. — Six defaunated adult Texel wethers (60-65 kg live weight), fitted with a rumen cannula and housed in individual, isolated pens, were individually fed equal amounts twice daily (8.00 and 16.30 h) at a constant daily rate of 50 g dry matter/kg body weight$^{0.75}$. Four were made protozoa-free by separating them from their dams within two hours after birth and putting them in isolated stalls (Williams and Dinusson, 1973). The other two sheep were made protozoa-free before the start of the experiment by emptying and washing the rumen of each one according to the procedure of Jouany and Senaud (1979*a*).

Experimental design.

The experiment consisted of four 2-month periods. In the first period, all six sheep were defaunated. In the second period, they were divided into three equal groups which were then inoculated in the rumen with one of the following three genera of protozoa : *P*, *E* or *I*. After 2 months (period 3), each group was subdivided and inoculated with a mixture of two genera of protozoa to obtain two sheep for each *P* + *E*, *P* + *I* and *E* + *I* combination in the rumen. In period 4, a conventional total fauna, containing *P*, *E*, *I*, *Dasytricha*, *Eudiplodinium* and *Epidinium* species, was restored in the rumen of two sheep. The microbial population in the rumen of each animal was observed daily during each period. Studies of digestion in all periods started one week after the microbial population in the rumen had reached a steady state.

Ciliate inoculum. — Using a micropipette under a binocular magnifying glass, pure ciliate inocula were prepared by sorting out a given genus from a mixture. The genus was then washed three times in Hungate's saline solution (1) at 41 °C and inoculated into the ventral pouch of the rumen.

Measurements. — Feed digestibility was determined twice in each sheep by total faecal collection for at least 5 consecutive days at the end of each period. Feed and faecal dry matters were calculated after a 24-hr drying period in a ventilated drying oven (80 °C), whereas the organic matter was determined by ashing samples at 550 °C for 6 hrs. Determination of lignocellulose (Van Soest, 1963), starch (Thivend, Mercier and Guilbot, 1965) and Kjeldahl nitrogen (1975) were carried out in the diet and faecal samples.

For 3 consecutive days, samples were taken from the rumen of each sheep just before the morning feeding (T0) and then 0.5 (T1/2), 1 (T1), 2 (T2), 3 (T3) and 5 (T5) hrs after feeding ; the pH was measured immediately after each sampling. The samples were then filtered through gauze. The filtrates were preserved in an orthophosphoric acid (5 p. 100 V/V) and a mercuric chloride (1 p. 100 W/V) solution for volatile fatty acid (VFA) analysis (10 ml of filtrate in 1 ml of preservative solution) or in a NaCl solution for preservation (12.5 p. 100 W/V) for ammonia analysis (5 ml of filtrate in 20 ml of NaCl solution) and stored at — 15 °C.

VFA analysis was carried out by gas-liquid chromatography using glass columns (2.16 mm inner diameter × 1.5 m). The column was packed with chromosorb W-AW-

(1) NaCl : 6 g ; $NaHCO_3$: 1 g ; KH_2PO_4 : 1 g ; $MgSO_4$: 0.1 g ; $CaCl_2$: 0.1 g in one liter of double-distilled water.

60-80 mesh coated with 10 p. 100 SP 1200 (Packard Inst. Soc.) (2) and 1 p. 100 orthophosphoric acid (weight basis). Glass wool, placed at the ends of the column, was soaked with a solution of 1 p. 100 (V/V) phosphoric acid in acetone. Each sample was centrifuged just before being injected into the top of the column. The moveable top was changed after every 100 injections to avoid ghosting peaks. A flame ionisation detector was used with the column.

The ammonia concentration in the rumen liquor was measured by the modified (Michel, 1971) method of Weatherburn (1967) and lactic acid by the method of Barker and Summerson (1941). Rumen gases were sampled (Jouany and Senaud, 1979*b*) and analyzed by gas-solid chromatography according to the modified (Jouany, 1978) method of Mc Arthur and Miltimore (1961).

Ciliate counting. — The rumen contents were filtered onto a wire mesh (1 mm wide). Ciliates in filtrates were counted daily in a Dolfuss counter dish 1 hr after the morning feeding.

Bacteria counting. — Bacterial concentrations were measured with a Coulter counter (TA) (3) according to the modified method of Hobson *et al.* (1966). Thus, the total bacteria counts, as well as the number of bacteria belonging to four different size-types, defined by Hungate (1966) and Eadie and Hobson (1962), were determined. Type A was composed of small bacteria (< 3 μm), including some cocci, small *Clostridium*, rods, *bacteroides* and *Butyrivibrio*. Type B (3 to 9.5 μm) was composed of large *Clostridium* and *Selenomonas*. Type C (9.5 to 18.5 μm) consisted of Eadie's « ovals », Quinn's « ovals » and *Ruminococcus* in rosette form ; type D consisted of very large bacteria (18.5 to 25 μm).

Statistical analysis. — The results were expressed as the mean values $\pm$ SD. Differences between the means were tested for significance by variance analysis (Snedecor and Cochran, 1971). They were considered significant when $P < 0.05$.

Results.

When the *P* + *E* species of protozoa were inoculated into the defaunated rumen, *P* was found to prevent *E* growth. In addition, when even a small quantity of *P* was inoculated into the rumen with the established *E* population as a single species of protozoa, the *E* disappeared. Similar observations have also been reported by other workers (Grain *et al.*, 1980 ; Senaud *et al.*, 1980).

Digestibility (fig. 1). — Dry matter and organic matter digestibilities were increased ($P < 0.05$) by inoculating *P* and decreased ($P < 0.05$) by inoculating *I* into a protozoa-free rumen. Results of digestibility with *E* alone and the *E* + *I* combination were inconclusive because each was obtained from only one animal. However, the positive effect of *E* alone on dry matter and organic matter digestibilities confirmed our previous findings (Jouany and Senaud, 1979*c*). No effect of *I* was seen in the *P* + *I* combination.

(2) Packard Instrument Society, 4-10, rue de la Grosse-Pierre, 94533 Rungis Cedex L 159, France.
(3) Coultronic France S.A., 14, rue Eugène-Legendre, 95580 Andilly, France.

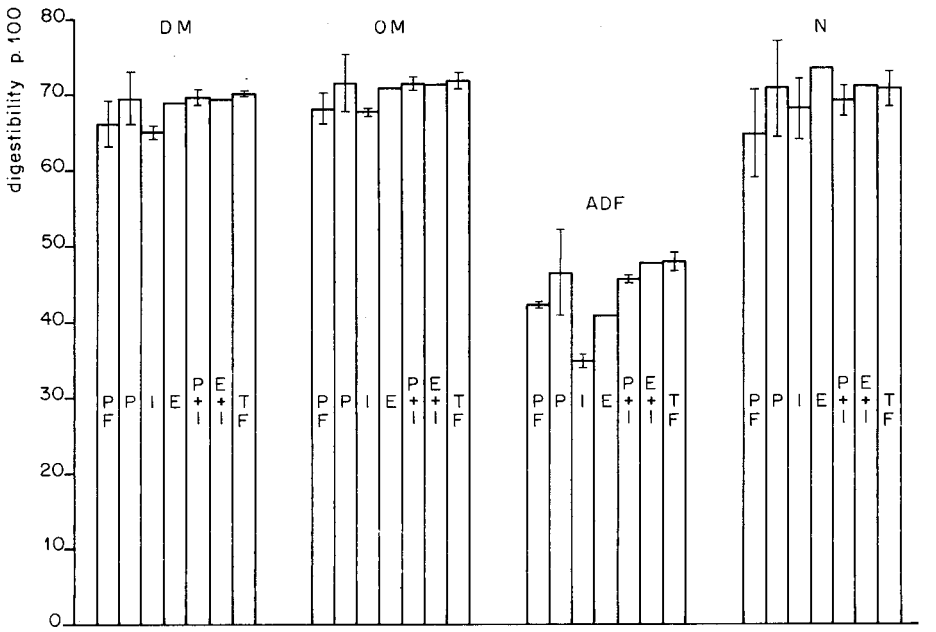

FIG. 1. — *Influence of the inoculated ciliate genus on dry matter* (DM), *organic matter* (OM), *acid detergent fiber* (ADF) *and nitrogen* (N) *digestibilities.*
PF = *protozoa-free* ; P = *Polyplastron* ; I = *Isotricha* ; E = *Entodinium* ; TF = totally faunted animals.

The presence of total fauna did not improve digestibility compared to that observed with other inocula containing *P* or *E*.

The pattern of lignocellulose digestibility was identical to that of organic matter, but variations in magnitude were greater. Thus, *I* decreased ADF digestibility by 7.4 units (*vs* 1.4 for organic matter) and the presence of total fauna increased it by 5.7 units (*vs* 4.0 for organic matter).

Ciliate inoculations always improved nitrogen digestibility. Yet, the individual variations observed were too great to show any significant divergencies between differently faunated sheep. Starch was always digested completely.

Rumen fermentation (table 1). — The inoculation of a monogeneric *P* fauna increased ($P < 0.05$) the VFA concentration and decreased ($P < 0.05$) the lactic acid concentration in the rumen samples. The mean pH value (obtained from samples taken at T1, T2, T3 and T5) was not altered. The composition of the VFA mixture was slightly affected ; the molar proportion of butyric acid (C4) was significantly increased ($P < 0.05$). However, the ratio of rumen gases (CO_2/CH_4) was modified. The ammonia nitrogen concentration (NH_3)-N in the rumen increased by as much as 75 p. 100.

Inoculation with *E* alone strongly affected rumen fermentation ; the molar proportion of acetic acid (C2) and C4 decreased by about 6 p. 100 and 16 p. 100, respectively, and that of propionic acid (C3) increased by 28 p. 100. The increase in C3 was confirmed by the rise in the CO_2/CH_4 ratio. The total VFA concentration increased by 11 p. 100

TABLE 1

Influence of the ciliate genera on rumen digestion parameters

	Animals							
	Protozoa-free (PF)	*Polyplastron* (*P*)	*Entodinium* (*E*)	*Isotricha* (*I*)	*P* + *I*	*E* + *I*	Totally faunated (TF)	Residual error
pH *	5.8 (^{ab}) (n = 72)	5.8 (^{ab})	5.9 (^{b})	5.7 (^{a})	5.9 (^{b}) (n + 48)	5.9 (^{b})	5.7 (^{a})	0.038
Volatile fatty acids (mM/l) *	83.2 (^{a}) (n = 72)	102.3 (^{b})	92.5 (^{cd})	86.4 (^{ad})	95.3 (^{bc}) (n = 48)	87.6 (^{acd})	91.3 (^{cd})	2.37
C2 (molar percentage) *	66.3 (^{a}) (n = 72)	66.6 (^{a})	62.1 (^{b})	62.5 (^{b})	65.7 (^{a}) (n = 48)	61.8 (^{b})	66.2 (^{a})	0.54
C3 (molar percentage) *	21.6 (^{ac}) (n = 72)	19.4 (^{a})	27.6 (^{b})	25.5 (^{bc})	20.8 (^{ac}) (n = 48)	23.2 (^{c})	15.4 (^{d})	0.65
C4 (molar percentage) *	9.3 (^{a}) (n = 72)	11.2 (^{b})	7.9 (^{c})	8.4 (^{ac})	10.9 (^{b}) (n = 48)	11.9 (^{b})	15.9 (^{d})	0.32
(NH_3) N (mg/l) *	149.4 (^{ac}) (n = 72)	261.9 (^{bd})	160.9 (^{c})	196.8 (^{bc})	271.1 (^{d}) (n = 48)	240.4 (^{e})	351.1 (^{f})	17.09
CO_2/CH_4 **	3.0 (^{a}) (n = 36)	2.8 (^{ac}) (n = 12)	3.7 (^{b}) (n = 12)	2.9 (^{a}) (n = 12)	2.1 (^{cd})	2.4 (^{acd}) (n = 12)	1.5 (^{d}) (n = 12)	0.15
Lactic acid (mg/l) ***	130.6 (^{a}) (n = 8)	91.0 (^{b}) (n = 6)	127.3 (^{a}) (n = 6)	222.7 (^{c}) (n = 6)	55.9 (^{b}) (n = 12)	164.1 (^{a}) (n = 6)	73.2 (^{b}) (n = 6)	19.02

24 samples were taken unless otherwise indicated.

Mean values and sample number (n) for T1, T2, T3 and T5 samples *, for T1 and T2 sampkes **, for T 1/2 samples ***. Values followed by different letters are significantly different ($P < 0.05$).

but lactic acid content was not modified. The considerable ciliate population differences between sheep (Senaud *et al.*, 1980) demonstrated that the (NH_3)-N concentration increased only when the *I* population attained very high numbers ($> 1 \times 10^6$/ml) (fig. 2).

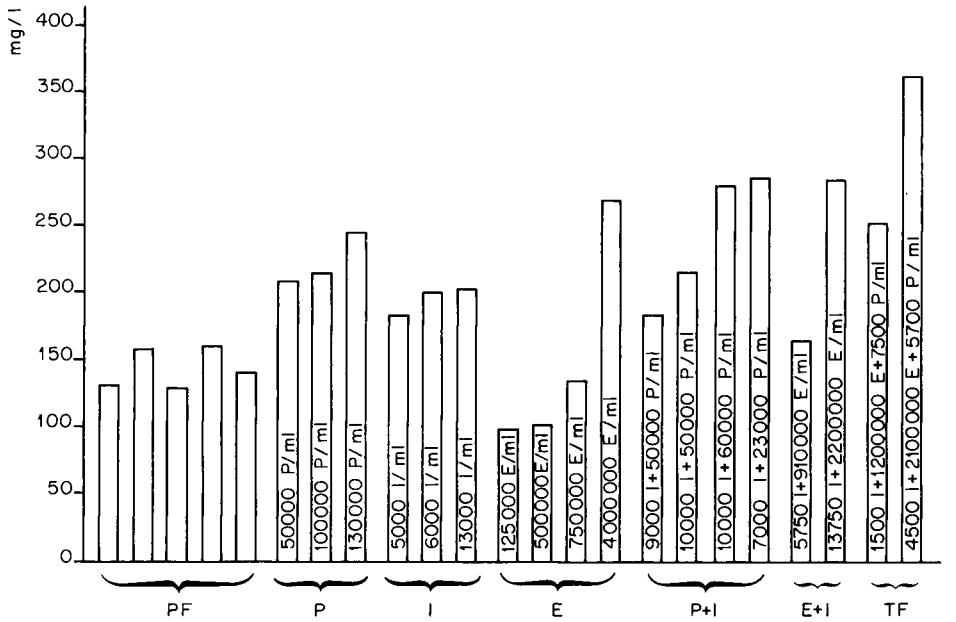

FIG. 2. — *Influence of ciliate genera and ciliate number present in the rumen on ammonia-nitrogen concentration* (means of T0, T1, T2, T3, T5 samples).

Inoculation of *I* alone greatly increased the concentration of lactic acid and decreased pH after feeding. The total VFA concentration was unchanged. In contrast, the composition of the VFA mixture was similar to that obtained with *E* alone but the gas formation was different. (NH_3)-N concentration increased.

The combination of *I* with either *P* or *E* had no noticeable effect on rumen fermentation compared with that obtained for *P* alone or *E* alone. There was an apparent increase in lactic acid concentration for the *E* + *I* combination and a decrease for the *P* + *I* combination. An observed increase in the molar proportion of C4, as well as a decrease in the molar proportion of C3 for the *E* + *I* combination, was closely related to the lower ratio of CO_2/CH_4 for this combination compared to *E* alone.

Compared to protozoa-free sheep, the presence of total fauna modified rumen fermentation in the same way as did *P* alone or the *P* + *I* combination, but the differences were significant ($P < 0.05$). This was also true for C3, C4 proportions and (NH_3)-N concentration, as well as for the CO_2/CH_4 ratio. Compared to *E* and the *E* + *I* combination, total fauna significantly decreased pH and greatly modified the VFA composition : the C3 molar proportion and the CO_2/CH_4 ratio decreased, while the C2 and C4 proportions increased ; also, the concentration of (NH_3)-N increased while that of lactic acid decreased.

Bacterial population (fig. 3). — Total bacterial concentration in the rumen varied little between the different groups of sheep ; it was 3.1×10^9 in defaunated, 3.3×10^9 in P-inoculated, 3.8×10^9 in *I*-inoculated, 3.0×10^9 in *E*-inoculated, 3.1×10^9 in $P + I$-inoculated and 3.2×10^9 in $E + I$-inoculated animals. It should be noted that when the *E* population grew from 0.4×10^6 to 2.2×10^6 per ml of rumen liquor, the total bacterial concentration changed slightly from 3.3×10^9 to 2.9×10^9 per ml of rumen liquor. In contrast, the distribution of the different size-type categories of bacteria varied (fig. 3). Compared to a protozoa-free rumen, the number of small bacteria (A) increased with *P* and *I* inoculations and decreased with *E* inoculation. The number of average-sized bacteria (B) was always higher in faunated than in defaunated sheep, except for the $E + I$ combination. The number of large bacteria (C) decreased in the faunated sheep, except for *I* alone. The largest bacteria (D), more numerous in the faunated sheep, showed a maximum with the $E + I$ combination.

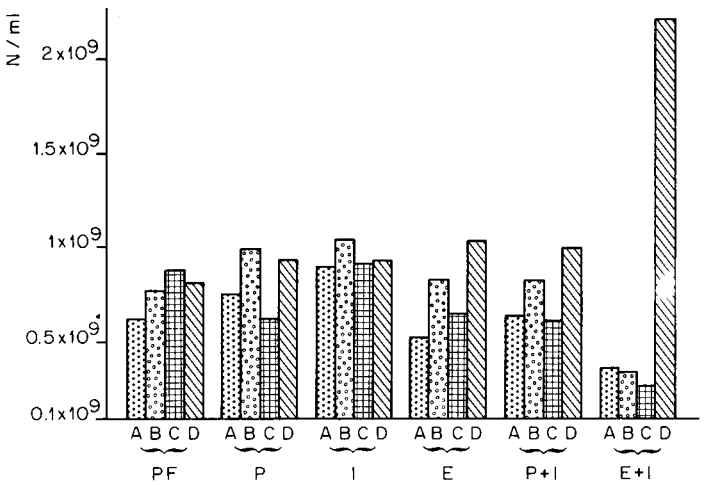

FIG. 3. — *Variations in different types of rumen bacteria in relation to inoculated ciliate genera.* Type A = very small bacteria (< 3 μm) ; Type B = small bacteria (3-9.5 μm) ; Type C = medium size bacteria (9.5-18.5 μm) ; Type D = large size bacteria (18.5-25 μm).

Discussion.

The number of animals used had to be limited given the experimental design and the fact that we wanted to use truly defaunated, mono or bi-inoculated sheep. Although weekly samplings were taken to check the fauna, no contamination appeared. This is worth noting, particularly as *Polyplastron multivesiculatum* is concerned, because, to our knowledge, this ciliate has never been studied alone *in vivo* (Coleman, Davies and Cash, 1972 ; Coleman and Laurie, 1977).

Digestibility. — The favourable effects of *P* and *E* inoculations on organic matter digestibility in the diet we used, containing 27 p. 100 starch and 39.5 p. 100 structural carbohydrates, were likely due to their glycolytic enzyme equipment. *In vitro*, Abou

Akkada *et al.* (1963) showed that *P* can break down different carbohydrates. *E* has an active amylase (Abou Akkada and Howard, 1960) ; its action on cellulose (Bonhomme-Florentin, 1975), hemicelluloses and pectin, however, is slight. *I* utilizes only certain cytoplasmic carbohydrates (fructose, glucose, sucrose, starch) (Oxford, 1951 ; Masson and Oxford, 1951 ; Heald and Oxford, 1953 ; Howard, 1959*a*, *b*). The inability of *I* to digest structural carbohydrates does not explain its depressive effect on lignocellulose digestibility, which is not caused by quantitative reduction of the bacterial population (fig. 3), but could be due to a drop in its cellulolytic activity following the presence of large amounts of lactic acid and to a greater drop in rumen pH after feeding (fig. 4).

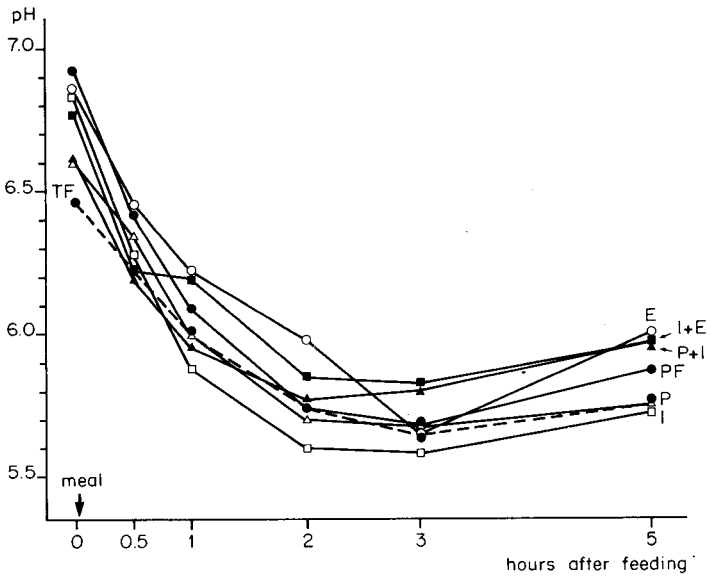

FIG. 4. — *pH of rumen content.* PF = *protozoa-free* ; P = *Polyplastron* ; I = *Isotricha* ; E = *Entodinium* ; TF = totally faunated animals.

The predatory ciliate action on bacteria (Coleman, 1975), which favours the metabolic activity of bacteria (Kurihara *et al.*, 1968 ; Thomas, 1973 ; Jouany and Senaud, 1978, 1979 ; Demeyer and Van Nevel, 1979), could be lower with *I* than with *E* ciliates (Gutierrez, 1958 ; Gutierrez and Davis, 1959). In the presence of *P* or *E*, the depressive action of *I* on digestion disappears. This might be explained by the great decrease in the number of *I* from 2×10^4 to 5×10^3 per ml of rumen liquor when inoculated with *P*, and from 1×10^4 to 6×10^3 per ml when inoculated with *E* (Senaud *et al.*, 1980). The lowest lactic acid concentrations in rumen liquor that were observed with the $P + I$ and $E + I$ combinations confirm this hypothesis.

Lindsay and Hogan (1972) showed that the amount digested in the large intestine increased with defaunation at the expense of that digested in the rumen. This shift in digestion therefore enables us to conclude that the improvement in organic matter digestibility observed with *P* or *E* would be greater in the rumen than in all the digestive tract.

Rumen digestion. — The presence of ciliates in this experiment affected VFA concentration and organic matter digestibility. It also increased the ammonia concentration in the rumen liquor, thus agreeing with most previously published results (see Jouany, 1978).

The increased butyric acid proportion in the VFA mixture, when the total fauna was present in the rumen, agrees with most other authors (e.g. Eadie and Mann, 1970 ; Clarke, 1977). Variations in the proportions of acetate and propionate are more difficult to compare with other reports, which are often contradictory (table 2), because

TABLE 2

Effect of ciliate inoculation on VFA *mixture (Bibliographic Data)*

Diet : R = Roughage C = Concentrate	Authors	Animals	Rumen fauna	VFA (mM/l)	Molar percentage		
					C2	C3	C4
R = 100	Jouany, 1978	sheep	Protozoa-free	78.0	72.6	18.6	4.6
			Polyplastron (*P*)	94.9	69.9	21.3	7.9
			Entodinium (*E*)	86.5	73.2	18.3	6.5
			P + *E*	86.3	71.9	18.9	5.6
			totally faunated	90.0	71.6	17.2	6.8
R = 80 C = 20	Luther, Trenkle and Burroughs, 1966	lambs	protozoa-free	69.7	50.8	30.8	15.5
			totally faunated	78.9	47.8	30.7	18.1
R = 66 C = 33	Abou Akkada and El Shazly, 1964	lambs	protozoa-free	40	77.5	14.9	7.6
			totally faunated	55	67.5	32.5	—
	Eadie and Gill, 1971	lambs	protozoa-free	67.4	75.4	15.9	8.2
			totally faunated	80	68.5	21.8	9.5
	Williams and Dinusson, 1973	calves	protozoa-free	88.9	64.3	23.0	10.7
			Entodinium	88.9	63.5	28.3	6.8
			Isotricha (*I*)	90.9	62.0	27.2	9.1
			E + *I*	90.0	61.7	22.6	14.2
R = 44 C = 56	Jouany, 1978	sheep	protozoa-free	103.0	58.8	35.1	4.2
			Polyplastron	117.6	56.3	31.1	11.3
			Entodinium	88.2	61.4	25.0	12.8
			P + *E*	116.8	58.9	29.9	9.6
			totally faunated	115.6	60.6	30.1	8.4
R = 50 C = 50	Christiansen, Kawashima and Burroughs, 1965	sheep	protozoa-free	63.3	62.3	21.4	15.2
			Entodinium	76.0	54.9	25.7	15.8
			Diplodinium (*D*)	70.1	49.5	25.0	21.5
			Isotricha	69.5	55.9	23.7	18.3
			Ophyoscolex (*O*)	66.4	54.7	24.9	18.4
			E + *D* + *I* + *O*	75.9	54.8	24.6	17.4
R = 40 C = 60	Males and Purser, 1970	sheep	protozoa-free	81.6	57.5	30.3	7.5
			totally faunated	74.4	61.4	23.3	12.1
R = 20 C = 80	Luther, Trenkle and Burroughs, 1966	lambs	protozoa-free	74.3	38.7	26.0	30.5
			totally-faunated	73.5	38.7	34.0	23.6
C = 100 restricted feeding	Eadie and Mann, 1970	heifers	protozoa-free	152	43	35	12
			totally faunated	113	64	11	20

rumen fauna vary with the diet, feeding level, physiological conditions and geographical origin of the animals. So, the present results seem to indicate that each genus studied had a specific action on the rumen fermentation pattern (table 1). To accurately evaluate results on the effect of ciliates on rumen digestion, it is therefore important to identify the qualitative and quantitative composition of rumen fauna in each study.

« Polyplastron », « Entodinium » and « Isotricha » relationships with bacteria. — Most previous reports have shown that bacterial concentration is greater in the protozoa-free rumen than in the faunated rumen, and that inoculation of ciliates into the former induces a decrease in bacterial number. In contrast, our results tend to show that bacterial number is not influenced by ciliate inoculation, even in the case of *E* when it reaches a high rumen concentration.

Like Eadie and Hobson (1962) and Kurihara *et al.* (1968), we noted that ciliate inoculation altered the number of different kinds of bacteria. However, we cannot conclude whether this was the result of selective predation by the inoculated ciliates, or competition for nutrients, or both. Nonetheless, the increase in the number of largest bacteria is likely due to the fact that they cannot be ingested by *I* which select their prey (Gutierrez, 1958), or by *E* for obvious reasons of size and shape (Coleman, 1964).

By using sheep with a solely *P* genus fauna in the rumen, we were able to determine *in vivo* the specific role of that ciliate species. To our knowledge, this is the first study of its kind. Of the three ciliate genera studied, *P* showed the greatest digestive activity, close to that of the total fauna. These results confirm those obtained in our previous studies (Jouany, 1978 ; Jouany and Senaud, 1979*c*) in which we compared the action of *P* and *E* on different carbohydrate diets. The preponderant action of *P* can be attributed to its ability to hydrolyse and ferment most carbohydrates, its predation on bacteria and other protozoal genera (Eadie, 1967) and its large size enabling it to ingest large amounts of the diet particles. However, it should be emphasized that the type of diet used is an important factor, and that complementary studies are needed to compare the roles of *P*, *E* and *I* genera in the digestion of diets rich in different structural and cytoplasmic carbohydrates.

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Résumé. A l'aide de six moutons dont le rumen est (1) défauné ou (2) mono-inoculé avec le genre *Polyplastron* (*P*) ou *Entodinium* (*E*) ou *Isotricha* (*I*), ou (3) inoculé avec deux d'entre eux *P* + *I* ou *P* + *E* ou *E* + *I*, ou (4) inoculé avec une faune conventionnelle, nous avons étudié l'influence de ces trois principaux genres de Protozoaires ciliés les plus fréquemment rencontrés sur la digestion d'un régime mixte contenant 50 p. 100 d'orge et 40 p. 100 de luzerne déshydratée. Les mesures ont porté sur la détermination de la digestibilité de la ration ainsi que sur les différents paramètres de la digestion au niveau du rumen. Nous avons également tenté de préciser l'influence de l'inoculation de chacun des trois genres étudiés sur la population bactérienne du rumen.

L'implantation de chacun des Ciliés dans les rumens défaunés a toujours été réussie. Par contre, il n'a jamais été possible d'obtenir l'état *P* + *E* car *E* a systématiquement et irréversiblement disparu en présence de *P* quel qu'ait été l'ordre des inoculations. La digestibilité de la matière organique et de l'ADF (acid detergent fiber) de la ration a été améliorée après l'inoculation de *E* et surtout de *P* dans les rumens non faunés (fig. 1). La présence d'une faune plus complexe n'a pas accentué cet effet. L'action de *I* a été significativement négative sur chacun des deux paramètres. La digestibilité de l'azote a toujours été accrue après l'implantation des ciliés, mais de manière non significative.

Au niveau du rumen, on a observé généralement une augmentation de la concentration en acides gras volatils (AGV) consécutive à l'inoculation des animaux défaunés (tabl. 1). Cette augmentation a été confirmée par une baisse de la valeur du pH sauf chez les animaux mono-inoculés avec *P*. La composition du mélange des AGV a été fortement influencée par la présence de *E* : le pourcentage molaire de l'acide propionique a augmenté alors que celui des acides acétique et butyrique a diminué. Les résultats sont corroborés par la réduction du rapport CH_4/CO_2 dans les gaz du rumen. Avec *P*, la proportion en acide butyrique a augmenté tandis que celle en acide propionique a diminué. Avec *I* inoculé seul, la teneur en acide lactique a fortement augmenté. L'adjonction de *I* à *P* ou *E* a peu modifié la composition du mélange des AGV. La présence d'une faune conventionnelle a orienté les fermentations de la même façon que *P* mais avec des différences qui sont devenues significatives. La concentration en N-(NH_3) du jus de rumen a toujours été plus importante chez les animaux faunés que chez les défaunés (fig. 2).

Enfin, nous avons constaté que la population des ciliés devait atteindre un certain seuil, évalué à environ $1,0 \times 10^6$/ml, pour que la concentration totale des bactéries du rumen diminue légèrement (fig. 3). La répartition qualitative des bactéries, évaluée selon leur taille, a été fortement influencée par l'implantation des Ciliés d'une manière dépendante de la nature du Cilié.

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