Effects of diet on the protozoa population in permeable continuous cultures of rumen contents

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1. Responses of the protozoa population to the composition and the components of the diet were studied in permeable continuous cultures of rumen contents.

2. In Expt 1 a study was made of responses to diets containing different combinations of rice straw, lucerne meal and mixed concentrates given to the cow supplying inocula for cultures. It was found that (1) when a diet devoid of concentrates was fed, entodiniomorphs decreased in numbers; (2) their numbers increased with the increase in the amount of concentrates; (3) holotrich numbers were hardly affected by the type of diet; (4) rice straw and lucerne meal were not essential for protozoa to survive in culture medium.

3. In Expt 2 responses were determined to diets containing different combinations of maize, maize starch, sugars (glucose–sucrose (1:1, w/w)), soya-bean meal and isolated soya-bean protein. The results suggested the following relationship between dietary component and protozoa population. (1) The diet rich in both starch and sugars sustains the increased numbers of protozoa on the whole. (2) The diet rich in starch and deficient in sugars decreases selectively Dasytricha and small species of Entodinium such as *Entodinium minimum* and *E. nanellum*. The other species of protozoa, especially large entodiniomorphs such as Ophryoscolex and Polyplastron, are maintained at relatively high levels. (3) The diet deficient in starch and abundant in sugars decreases general entodiniomorphs except small species, while the numbers of Holotrichs are kept at relatively high levels.

Although much indispensable information has been accumulated about the dietary factors that influence the protozoa population in the rumen (Warner, 1965; Hungate, 1966; Eadie & Mann, 1970), more detailed studies are necessary for the complete understanding of the complex relationship between the diet and the protozoa population.

Recent advances in studies on in vitro continuous culture of the rumen contents provided three types of culture systems which permit the maintenance of protozoa numbers at an appropriate level for an extended period (Abe & Kumeno, 1973; Weller & Pilgrim, 1974; Hoover, Crooker & Sniffen, 1976). With one of these systems, Hoover, Knowlton, Stern & Sniffen (1976) demonstrated that protozoa numbers in the culture were affected by the change in the composition and the particle size of diet. If protozoa populations in these continuous cultures respond sensitively to the change in dietary composition, as was found in the rumen (Abe, Shibui, Iriki & Kumeno, 1973), a great deal of useful knowledge on the interrelationship between the diet and protozoa population will be provided by in vitro culture studies.

The present paper describes the influence of the composition and the components of the diet on the protozoa population in semipermeable continuous cultures of rumen contents.

EXPERIMENTAL

Continuous-culture experiments

Inocula for cultures were obtained from a 600 kg cow which was fed daily 3 kg chopped rice straw, 3 kg dehydrated lucerne-meal pellets (LMP) and 1·5 kg mixed concentrates (MCF), in two portions at 08.00 and 16.00 hours. The MCF consisted of (g/kg) 372 cereal grain (maize and sorghum), 288 bran (wheat bran and defatted rice bran), 274 vegetable oil meal

Table 1. *Composition (g/d) of diets used in Expts 1 and 2*

Expt 1				Expt 2					
	Ingredient				Ingredient				
Diet	Ground rice straw	Lucerne-meal pellet	Mixed concentrates	Diet	Ground maize	Maize starch	Sugar*	Soya-bean meal	Isolated soya-bean protein
D1	200	200	100	D7	150	—	—	80	—
D2	—	200	—	D8	150	—	—	—	50
D3	200	200	—	D9	—	100	30	80	—
D4	—	200	100	D10	—	100	30	—	50
D5	—	—	100	D11	—	100	—	—	50
D6	—	—	250	D12	—	—	100	—	50

* Glucose–sucrose (1:1, w/w).

(mainly, soya-bean meal and rapeseed meal), 40 molasses and 26 minerals, and contained (g/kg) 185 crude protein (CP; nitrogen $\times 6{\cdot}25$) and 65 acid-detergent fibre. Rumen contents were collected through a rumen fistula approximately 5 min after the morning feed and strained through two layers of gauze. Before using the strained fluid as inocula, protozoa population was estimated to confirm that the protozoa count ranged from 2×10^5 to 4×10^5/ml. Fluid samples with protozoa counts outside this range were not used.

The continuous-culture device and the operating procedure have been described by Abe & Kumeno (1973). The urea content of salivary buffer solution was 2·5 g/10 l in Expt 1, and was increased to 7·5 g/10 l in Expt 2. The dilution rate of the contents of the culture vessel was fixed to 0·02/h throughout the present studies. The diets were administered to the culture vessel at half the daily amount at 08.30 hours and at 16.30 hours. All cultures were duplicated.

Expt 1. Protozoa responses to the diet which was given to the cow supplying inocula were determined in continuous cultures for 80 h each. Six combinations of dietary components, rice straw, LMP and MCF, were tested in Expt 1, and the daily amounts are shown in Table 1. Rice straw and MCF were ground in a Wiley mill through 1 mm mesh screen, and the ground rice straw (GRS) was soaked in salivary buffer solution before feeding. GRS not soaked in advance exerted deleterious effects on protozoa due to its inclusion of air. LMP was fed as in a pelleted form.

Expt 2. In Expt 2, the effects of soya-bean meal (SBM) and isolated soya-bean protein (ISP; CP content 770 g/kg) as a protein source, and the effects of maize, maize starch and sugars (glucose–sucrose (1:1, w/w)) as a carbohydrate source on protozoa population were determined in cultures for 80 h each. The six combinations of maize, maize starch, sugars and protein sources, and their daily amounts are given in Table 1. Maize and SBM were ground to pass 1 mm mesh screen.

Analytical methods

For all cultures, samples of effluents from the culture vessel were taken daily at 2 h intervals starting immediately before the morning feed at 08.30 hours until just before the evening feed at 16.30 hours. The samples obtained were strained through two layers of gauze, and part of the strained fluid was used for the determination of protozoa counts and the production of volatile fatty acids (VFA). The possibility that straining of effluents may affect the protozoa counts was ignored, for straining increase the ease and accuracy of counting.

Protozoa counts were estimated as described by Abe *et al.* (1973). Total VFA levels were determined as described previously (Abe & Kumeno, 1973), and VFA compositions were

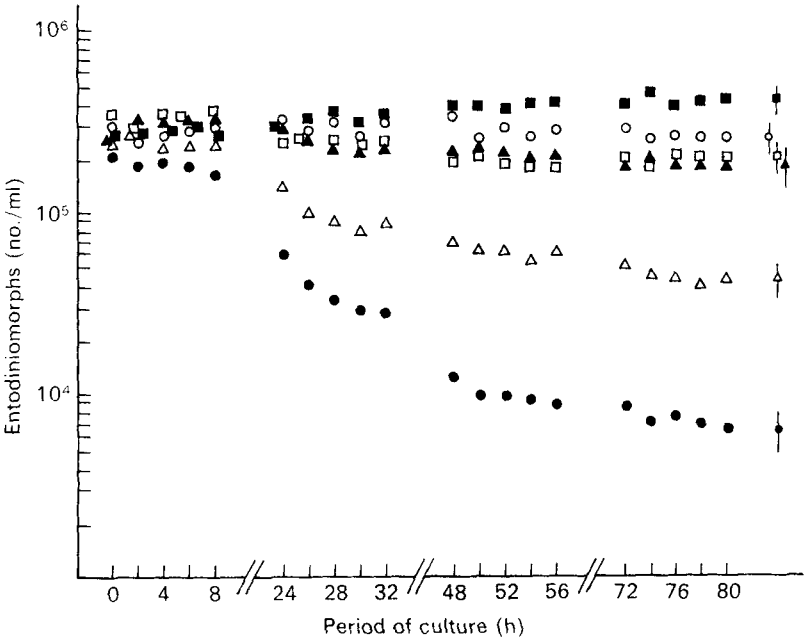

Fig. 1. Expt 1. Changes in entodiniomorph numbers in cultures given diets: D1 (○), D2 (●), D3 (△), D4 (▲), D5 (□), D6 (■). The compositions of diets D1–6 are shown in Table 1. The vertical bars represent the standard errors of protozoa counts at the end of duplicate 80 h cultures.

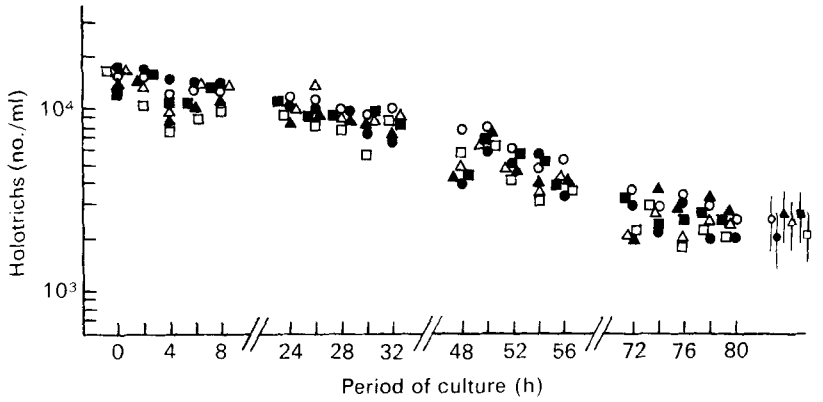

Fig. 2. Expt 1. Changes in holotrich numbers in cultures given diets: D1 (○), D2 (●), D3 (△), D4 (▲), D5 (□), D6 (■). The compositions of diets D1–6 are shown in Table 1. The vertical bars represent the standard errors of protozoa counts at the end of 80 h cultures.

determined using a gas–liquid chromatograph (Type JGC-1100 FEP; Japan Electron Lab. Co., Tokyo) equipped with a flame-ionization detector and a 1·8 m glass column, 3 mm diameter, packed with C-22 (60–80 mesh) adsorbed 100 g DEGS (Nihon Chromato Works, Ltd., Toshima, Kitaku, Tokyo)/kg support and 20 g H_3PO_4/kg DEGS. Samples were prepared by centrifuging the strained fluid after the addition of 1 ml 0·5 M-H_2SO_4/2 ml fluid. Operating conditions of the gas–liquid chromatograph were as follows: inlet temperature 163°, column temperature 131°, detector temperature 160°, nitrogen gas flow-rate 38 ml/min, air flow-rate 350 ml/min, hydrogen gas flow-rate 32 ml/min.

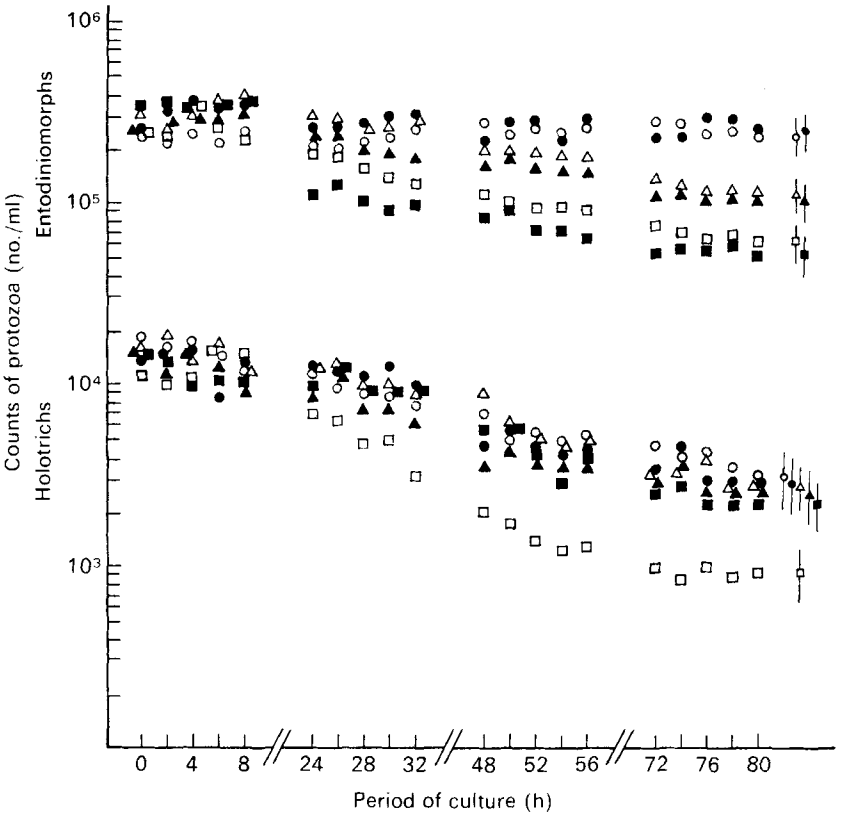

Fig. 3. Expt 2. Changes in the numbers of entodiniomorphs and of holotrichs in cultures given diets: D7 (○), D8 (●), D9 (△), D10 (▲), D11 (□), D12 (■). The compositions of diets D7–12 are shown in Table 1. The vertical bars represent the standard errors of protozoa counts at the end of duplicate 80 h cultures.

RESULTS

Changes in the numbers of entodiniomorphs and holotrichs in Expt 1 are shown in Figs 1 and 2 respectively. In the culture given the diet consisting of 200 g GRS, 200 g LMP and 100 g MCF (diet D1), entodiniomorphs were maintained at approximately the original level of 30×10^4/ml for 80 h. When a single portion of 200 g LMP (diet D2) was given, they decreased rapidly to a level of 7×10^3/ml. The addition of 200 g GRS and 200 g LMP (diet D3) alleviated the decrease, but the numbers were reduced to a level of 3×10^4/ml. When 200 g LMP and 100 g MCF (diet D4) was given, the numbers were maintained at a level of 20×10^4/ml, but nearly the same effect was also derived from feeding 100 g MCF alone (diet D5). Increasing the amount of MCF to 250 g/d (diet D6), increased the level from 30×10^4 to over 40×10^4/ml in an 80 h culture.

Although holotrichs decreased in all cultures, no apparent differences were seen in the change in their numbers among diets tested in this experiment. Protozoa were very active and no particular change in the constitution of fauna was apparent in Expt 1, except the decrease in holotrich numbers.

Responses of protozoa in Expt 2 are shown in Fig. 3. When 150 g maize was given with SBM (diet D7) or with ISP (diet D8), entodiniomorphs maintained their numbers at the original level for 80 h, and no difference was observed between the effects of diets D7 and D8. When a mixture of 100 g starch, 15 g glucose and 15 g sucrose was given with SBM

Table 2. *Expt 2. Numbers of Isotricha, Dasytricha and large,* middle† and small‡ entodiniomorphs (classified according to size) in cultures§ given synthetic diets (diets D10–12)*‖

Period after the start of culture (h)	Holotrichs (no. × 10^{-2}/ml) in culture given diets						Entodiniomorphs (no. × 10^{-4}/ml) in culture given diets								
	D10		D11		D12		D10			D11			D12		
	Isotricha	Dasytricha	Isotricha	Dasytricha	Isotricha	Dasytricha	Large (× 10^{-2})	Middle	Small	Large (× 10^{-2})	Middle	Small	Large (× 10^{-2})	Middle	Small
0	60·8	84·1	56·3	79·5	68·3	67·5	6·3	23·8	11·2	9·8	26·8	11·9	11·3	27·3	12·1
2	48·3	78·8	41·3	69·0	59·6	74·5	5·7	26·4	12·1	9·2	29·7	11·8	8·3	32·2	13·3
4	77·7	62·9	53·7	70·3	52·5	52·8	7·5	23·6	12·5	10·1	30·2	13·6	8·3	23·2	13·1
6	51·0	71·3	43·8	85·5	41·5	67·6	8·9	28·8	12·2	10·7	31·8	11·3	9·0	23·8	10·9
8	40·5	52·5	58·5	72·8	42·4	50·2	8·5	21·3	11·3	12·0	28·1	10·9	7·5	19·9	11·4
Average	55·7	69·9	50·7	75·4	52·9	62·5	7·4	24·8	11·9	10·4	29·3	11·9	8·9	25·3	12·2
SE	6·4	5·6	3·4	3·1	5·1	4·7	0·6	1·3	0·2	0·5	0·9	0·5	0·7	2·1	0·5
72	9·8	20·7	10·5	—	8·7	17·9	8·3	10·9	4·0	23·3	11·0	0·7	0·5	1·9	4·4
74	12·0	24·6	8·3	—	10·7	19·1	7·3	9·0	4·4	21·6	7·9	0·4	0·6	2·4	4·1
76	10·3	16·7	12·8	—	10·9	12·8	7·0	8·8	3·8	23·5	7·5	0·2	0·5	2·8	3·7
78	9·8	18·4	9·0	—	9·8	18·7	8·0	9·2	4·2	22·5	6·4	0·4	0·3	3·2	5·4
80	7·5	21·3	9·8	—	9·3	17·2	8·6	9·2	3·6	24·0	6·5	0·5	0·8	2·0	3·6
Average	9·9	20·3	10·1	—	9·9	17·1	7·8	9·4	4·0	23·0	7·9	0·4	0·5	2·5	4·2
SE	0·7	1·4	0·8	—	0·4	1·1	0·3	0·6	0·1	0·4	0·8	0·03	0·03	0·2	0·3

* Mainly, Ophryoscolex and Polyplastron.
† Mainly, *Entodinium caudatum, E. simplex, E. vorax, E. furca, E. longinucleatum, Diplodinium dentum, D. affine* and *Eudiplodinium neglectum.*
‡ Mainly, *E. minimum* and *E. nanellum.*
§ For details, see p. 256.
‖ Diet D10 contained both starch and sugars, D11 lacked sugars and D12 lacked starch; for details, see Table 1.

(diet D9) or with ISP (diet D10), their numbers decreased to a level of 10×10^4/ml in 80 h, but no significant differences were seen between the effects of diets D9 and D10. The results indicated that SBM did not contain any specific factors other than those which might be contained in ISP and also that ground maize possessed some undetermined factor(s) which were necessary for the in vitro growth of entodiniomorphs. When 100 g starch or 100 g sugar mixture (glucose–sucrose (1:1, w/w)) was given with ISP (diets D11 and D12 respectively), entodiniomorphs decreased to about half the numbers found in the culture given diet D10 for 80 h. Although no remarkable differences were observed between the effects of diets D11 and D12 on entodiniomorph numbers, some divergences were detected in the constitution of fauna between cultures given the two diets.

In Expt 1 in which diets of a 'natural' type were given in all cultures, no apparent differences were observed in holotrich numbers. However, some differences were detected in their numbers in Expt 2. More rapid decrease occurred in the culture given the synthetic diet without sugars (diet D11). Table 2 shows the change in numbers of Isotricha and Dasytricha, in each of three cultures given diets D10 (starch and sugars), D11 (no sugars) or D12 (no starch). Results are given only for periods of 8 h at the start and at the end of the 80 h culture period. No difference was observed in the decreased rate of both the species of holotrichs between diets D10 and D12. But in the culture given diet D11, Dasytricha disappeared approximately 50 h after the start, although Isotricha remained for 80 h at the same level as with the other two diets, indicating that Isotricha can ferment both starch and sugars, while Dasytricha relies principally on sugars to supply energy.

Table 2 also shows the numbers of entodiniomorphs in cultures given diets D10–12. In this Table, they are classified into three groups of 'large', 'middle' and 'small' size. The 'large' group included primarily Ophryoscolex and Polyplastron, the 'small' group included small species of Entodinium such as *Entodinium minimum* and *E. nanellum*. Most of remaining entodiniomorphs were included in the 'middle' group, and *E. caudatum*, *E. simplex*, *E. vorax*, *E. furca*, *E. longinucleatum*, *Diplodinium dentatum*, *D. affine* and *Eudiplodinium neglectum* were always discriminated. However, the proportion of individual species of entodiniomorphs in these groups were not identified. The 'large' group maintained their original numbers in the culture given diet D10 containing both starch and sugars, while the other groups decreased to about one-third the original numbers. In the culture given diet D11 lacking sugars, an increase of over twofold in the numbers of large entodiniomorphs and a decrease in those of small species of Entodinium were observed in the 80 h culture. In the culture given diet D12 (no starch), large entodiniomorphs decreased rapidly, and of the remaining entodiniomorphs there was a proportionate increase in small species of Entodinium. The results indicated that most small species of Entodinium are primarily sugar fermentors, and that starch is essential to the other entodiniomorphs. Furthermore, the results suggested that undetermined factor(s) which were present in ground maize and assumed to be indispensable to entodiniomorphs, are not necessary at least for the large species identified in the present work.

Table 3 shows the total VFA levels and the compositions in effluents sampled just before the morning feed on the first and the last days of each culture experiment. The values at 0 h were regarded as both the basal levels and basal composition of VFA in the rumen inoculum used in each culture. The differences observed between the values at 0 h and 72 h were considered as a reflection of some shift in microbial population, especially in protozoa population, in each incubation. Tendencies for a decrease in the molar proportion of acetic acid and for an increase in that of butyric acid were consistently observed in all cultures, as has been already reported (Abe & Kumeno, 1973; Weller & Pilgrim, 1974). In Expt 1 such a trend grew more pronounced as the amount of MCF in the diet increased, and in cultures given diets without MCF (diets D2 and D3) the change in VFA composition was relatively

Table 3. *Expts* 1 *and* 2. *The production and the composition of volatile fatty acids (VFA) in cultures* given various experimental diets*

(Mean values with their standard errors for duplicate analyses)

Period after the start of culture (h)		Diet† D1 Mean	D1 SE	D2 Mean	D2 SE	D3 Mean	D3 SE	D4 Mean	D4 SE	D5 Mean	D5 SE	D6 Mean	D6 SE
	VFA (mmol/l)	95·0	3·1	91·2	3·1	88·4	5·4	103·8	7·0	108·0	7·6	88·0	3·3
	Composition (mmol/mol):												
0	Acetic	667	6	640	17	653	15	685	13	653	23	665	23
	Propionic	136	12	145	40	128	9	124	16	142	15	126	17
	Isobutyric	52	46	46	7	51	11	47	12	45	8	40	9
	Butyric	100	3	127	11	107	13	123	7	113	6	125	12
	Isovaleric	32	11	29	12	42	5	7	4	32	7	30	8
	Valeric	13	6	13	3	19	9	16	1	15	8	14	7
	VFA (mmol/l)	128·4	10·9	114·4	5·1	113·0	6·4	127·7	8·2	96·8	3·6	137·3	1·9
	Composition (mmol/mol):												
72	Acetic	601	17	637	30	648	16	572	34	564	17	560	32
	Propionic	114	7	142	24	153	20	139	27	120	10	106	15
	Isobutyric	30	4	24	4	22	4	23	4	34	9	26	5
	Butyric	175	23	139	16	126	18	199	17	183	12	220	28
	Isovaleric	47	6	27	13	26	12	30	7	61	3	46	22
	Valeric	33	2	31	11	25	10	37	1	38	9	42	16

Period after the start of culture (h)		Diet† D7 Mean	D7 SE	D8 Mean	D8 SE	D9 Mean	D9 SE	D10 Mean	D10 SE	D11 Mean	D11 SE	D12 Mean	D12 SE
	VFA (mmol/l)	88·1	2·4	93·7	2·7	96·9	0·3	94·2	3·3	102·1	7·6	95·6	2·1
	Composition (mmol/mol):												
0	Acetic	667	18	678	15	712	24	708	21	700	16	705	7
	Propionic	115	4	116	6	112	12	119	7	103	7	112	16
	Isobutyric	43	3	28	4	27	4	27	13	37	6	29	6
	Butyric	103	12	120	13	98	9	87	15	103	7	112	16
	Isovaleric	54	9	41	6	39	9	48	12	44	8	46	15
	Valeric	18	8	17	12	12	2	11	4	14	1	13	2
	VFA (mmol/l)	154·4	4·1	143·8	5·2	162·3	4·6	120·5	1·2	117·3	10·2	103·2	7·3
	Composition (mmol/mol):												
72	Acetic	512	16	526	25	450	36	466	24	436	27	397	20
	Propionic	139	15	99	12	177	4	97	18	158	29	138	18
	Isobutyric	28	7	19	8	19	3	19	8	27	6	22	9
	Butyric	219	14	259	43	264	19	360	38	285	26	383	54
	Isovaleric	59	7	54	15	45	8	30	11	58	15	34	11
	Valeric	43	1	43	7	45	2	28	7	36	3	26	16

* For details, see p. 256.

† Compositions of diets D1–12 are shown in Table 1.

smaller. In the cultures given synthetic diets containing starch, or sugars, or both in Expt 2, the decrease in acetic acid was followed by a pronounced increase in butyric acid. The results seemed to agree with the observation in the rumen of cattle given purified diets with varying carbohydrate sources (Ørskov & Oltjen, 1967).

DISCUSSION

In the present studies with the continuous-culture system with dialysis (Abe & Kumeno, 1973), the protozoa population altered sensitively in response to the variation in dietary composition. The results of Expt 1 can be summarized as follows: (1) when a diet devoid of concentrates was fed, the numbers of entodiniomorphs decreased; (2) their numbers increased with increase in the amount of concentrates; (3) rice straw and lucerne meal were not essential for protozoa to survive; (4) the type of diet affected holotrich numbers to a lesser extent than entodiniomorph numbers. This series of relationships between dietary composition and protozoa population is in good accordance with that obtained previously with cows (Abe *et al.* 1973).

The results of Expt 2 suggested the presence of unknown factor(s) in ground maize, supporting the growth of smaller entodiniomorphs, which were not supplied by maize starch and protein. Such factor(s) are not necessary for large species (mainly, Ophryoscolex and Polyplastron in the present work), and perhaps for holotrichs. The factor(s) might be carbohydrates other than those examined in the present work, because some Entodinia have been demonstrated to possess α-glucosidase (maltase) (*EC* 3.2.1.20) activity together with the activity of α-amylase (*EC* 3.2.1.1) (Abou Akkada & Howard, 1960). However, there have been no reports of any individual species of entodiniomorphs which cannot ferment at least one of the three carbohydrates used in the present studies (Hungate, 1966).

Recently, some sterols, especially β-sitosterol, were shown to be essential for mixed species of Entodinium to grow in in vitro cultures with limited growth of bacteria (Hino, Kametaka & Kandatsu, 1973). But it is questionable if the difference in the numbers of entodiniomorphs between cultures given diets D7 or D8 (maize with SBM or ISP respectively) and those given diets D9 or D10 (starch and sugars with SBM or ISP) is fully explained by the difference in dietary sterol contents, because no appreciable difference was observed between cultures given diets D9 and D10. The growth of Entodinia was guaranteed by just a trace amount of β-sitosterol (Hino & Kametaka, 1974), and a considerable amount of this substance is contained not only in maize but also in SBM.

Hoover *et al.* (1976) reported that a decrease in the particle size of the diet resulted in reduced numbers of protozoa (mainly, entodiniomorphs) in their culture system, suggesting that dietary ingredients with fine physical properties may contribute to a decrease in protozoa numbers in cultures given semi-purified diets. Also in the present work, the decrease in entodiniomorph numbers in cultures given synthetic diets could be attributed to the fine physical property of the diets, for the particle sizes of starch and sugars were much smaller than that of maize and SBM which were ground to pass a 1 mm mesh screen. Furthermore, a considerable improvement observed in entodiniomorph numbers in the culture given diet D3 (GRS with LMP) as compared with that given diet D2 (LMP alone) might reflect some improvement in physical conditions or dry matter with the additional GRS.

Even if the effects of physical aspects of diet on protozoa numbers are not indeed ignored, the present results suggest that the type and amount of readily fermentable carbohydrates in diet will decide primarily the protozoa population. Considering the evidence obtained from the present experiments, the relationship between the dietary components and protozoa population can be summarized as follows: (1) on the whole, the diet abundant in both starch and sugars sustained increased numbers of protozoa; (2) the diet abundant in starch

and deficient in sugars decreased selectively Dasytricha and some species of small Entodinium, while the other species of protozoa, especially large entodiniomorphs such as Ophryoscolex and Polyplastron, were maintained at relatively high levels; (3) the diet deficient in starch and abundant in sugars decreased general entodiniomorphs, except some species of small Entodinium, while the numbers of holotrichs were kept at relatively high levels. These relationships are, in general, supported by information reported in the literature by many other workers on the biochemistry of single species of protozoa. Holotrichs are an active fermentor of soluble sugars (Oxford, 1951). Dasytricha cannot attack starch, but Isotricha can ferment it (Heald & Oxford, 1953; Mould & Thomas, 1958; Howard, 1959), though both the holotrich species contain α-amylase (Mould & Thomas, 1958). On the other hand, general entodiniomorphs are an active fermentor of starch, and reported to have very limited ability to use soluble sugars (Abou Akkada & Howard, 1960), except some very small species of Entodinium (Sudgen, 1953). Polyplastron, one of the large entodiniomorphs identified in this work, has been shown not to be able to use soluble sugars (Sudgen, 1953).

In the present studies, responses of the protozoa population to cellulose were not determined, because there was no appreciable response of protozoa either in Expt 1 reported here or in the rumen as reported by Abe *et al.* (1973). Many workers have observed that many species of protozoa ingest finely powdered cellulose or fibrous particles, and some species have been demonstrated to have a cellulose-digesting ability (Abou Akkada, Eadie & Howard, 1963). However, it has not been shown that they obtain energy from the digestion of cellulose even in the presence of more fermentable carbohydrates such as starch and sugars. Judging from the results of this work, it appears that the amount and source of cellulose can affect protozoa population to a much lesser extent than starch and sugars.

The response of protozoa population to the nature and quantity of the nitrogen source was not determined in detail in the present work as was that to carbohydrate. We have tried to compare the effects of ground maize alone, maize starch with zein, and starch with urea on the protozoa population. However, protozoa responses were not clear because in the cultures given these diets dialysis tubes placed inside the culture vessel were destroyed. The semipermeable membrane used for these dialysis tubes was Naturin sausage casing (no. 30/32; Becker Co., Weinheim, Germany) which was denaturized animal gut containing 736 g CP/kg, by analysis. The most rapid destruction occurred when maize starch was fed with urea, suggesting that microbial digestion of the proteinous membrane occurred when the diet lacked protein, especially a readily digestible protein. For this reason, fairly large amounts of SBM or ISP were included in all the diets tested in Expt 2, and the urea content of salivary buffer was increased to 7·5 g/10 l.

The rumen protozoa have an active proteinase (Abou Akkada & Howard, 1962), and it has been suggested that non-bacterial protein may be a source of N for rumen protozoa, and that they may ferment protein to obtain energy (Williams, Davis, Doetsch & Gutierrez, 1961). However, it has been demonstrated that rumen protozoa utilize bacteria as a N source both by in vitro culture studies (Hungate, 1966) and by the in vivo experiments with ^{15}N (Abe & Kandatsu, 1968). It is considered that the dietary N source cannot be deficient to protozoa when normal numbers of rumen bacteria are maintained. Therefore, it appears that the dietary N source affects protozoa population to a much less extent than carbohydrate source, as far as 'natural' diets with an appropriate level of protein are concerned.

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