The relationship between rumen bacterial growth, intake of dry matter, digestible organic matter and volatile fatty acid production in buffalo (*Bos bubalis*) calves

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1. The production rates of bacteria in the rumen of buffalo (*Bos bubalis*) calves were estimated using an isotope-dilution technique. A series of fifteen experiments was done with animals given green maize and nine experiments with animals given cowpea (*Vigna unguiculata*).

2. The turnover time ranged from 205 to 567 min in the group given green maize and from 330 to 648 min in animals offered cowpea. The production rates of bacteria were (mean $\pm$ SE; g/d) $145 \cdot 77 \pm 7 \cdot 240$ and $237 \cdot 09 \pm 11 \cdot 847$ in animals given green maize and cowpea respectively.

3. There was a significant correlation between bacterial production rates and dry matter intake, digestible organic matter and total volatile fatty acids formed in the rumen.

4. Regression equations obtained for the two foodstuffs were different suggesting that the bacterial growth rate may vary depending upon the quantity and quality of foodstuff digested and possibly the ratio nitrogen:energy of the foodstuff.

Ruminants meet their protein or amino acid requirements from the dietary proteins and rumen micro-organisms which leave the rumen and are digested in the small intestine. These micro-organisms can degrade dietary proteins and may utilize some of the degradation products for their own protein synthesis. Microbes in the rumen can also utilize non-protein nitrogenous compounds and can convert dietary proteins of low biological value into microbial proteins which are of higher biological value (Bergen, Purser & Cline, 1967). It is important to know the extent of microbial growth for any particular dietary regimen in order to assess its effectiveness for animal production.

In vitro and in vivo experiments have been done to estimate microbial growth in the rumen. In vitro techniques (Henderickx, 1959, 1961; Walker & Nadar, 1968; Van Nevel, Demeyer & Henderickx, 1974) and theoretical calculations (Hungate, 1966; Baldwin, Lucas & Cabrera, 1970; Forrest & Walker, 1971; Leng 1972) have been used by many workers. The validity of both in vitro and theoretical values will depend upon their confirmation by in vivo experiments. Attempts were made by various workers to estimate microbial protein synthesis in vivo using ^{15}N (Mathison & Milligan, 1971) and ^{35}S (Roberts & Miller, 1969; Harrison, Beever & Thomson, 1972; Singh, Varma, Verma, Lal & Ranjhan, 1973; Singh, Verma, Varma & Ranjhan, 1974*a*, 1974*b*) and ^{14}C (Singh, Varma, Verma & Ranjhan, 1974). Different markers, e.g. nucleic acids (Smith, 1969; Smith & McAllan, 1971), diaminopimelic acid (DAPA) (Hutton, Bailey & Annison, 1971; Singh, Verma, Varma & Ranjhan, 1976) and vitamin B_{12} (Weller, Gray & Pilgrim, 1958), have been used to assess microbial protein synthesis in the rumen. The results of several studies involving twenty-seven diets and utilizing DAPA as a bacterial marker for bacterial cell growth in the rumen have been reviewed by Thomas (1973). The cell yield varied from $15 \cdot 3$ to $51 \cdot 1$ g N/kg organic matter fermented.

The bacterial growth is variable and may be influenced by the nature of the micro-organisms, type of substrate in the rumen and the dilution rate (Hogan & Weston, 1971; Thomas, 1973). This paper describes a detailed study of in vivo estimations of bacterial

production rates in the rumen, using an isotope-dilution technique with ^{14}C and ^{35}S, for two different foodstuffs and also determination of the relationship between bacterial production and dry matter (DM) intake, digestible organic matter (DOM) and volatile fatty acid (VFA) production.

EXPERIMENTAL

Animals and feeding regimen

Three male Murrah buffaloes (*Bos bubalis*) approximately 2 years of age, each fitted with a rumen cannula, were used in these studies. The animals were housed singly in metabolism cages. In the first series of experiments each animal was given 15–20 kg chopped green maize (*Zea mays* L. var. Ganga hybrid 2) daily, whereas in the second series of experiments 25–30 kg cowpea (*Vigna unguiculata*)/d was fed to each animal. The amount of uneaten food was measured to estimate intake. The calves were subjected to a pre-experimental feeding period of 5 weeks during which they received their ration once daily, and thereafter they received their daily ration in twelve equal amounts at 2 h intervals for a period of 3 weeks. The ration remaining, if any, at the end of each 2 h period was removed and weighed. The samples of the food offered and the uneaten food were collected daily for analysis. Digestibility trials of 7 d duration were also done to estimate DOM. The analysis of food and faeces was done according to the methods of the Association of Official Agricultural Chemists (1960).

Estimations of VFA production rates

VFA production rates were estimated by the single-injection isotope-dilution technique as described previously (Chaturvedi, Singh & Ranjhan, 1973).

Preparation of labelled mixed bacteria

Rumen bacteria from the experimental calves were labelled either with ^{35}S or ^{14}C by in vitro incubation as described previously (Singh, Verma *et al.* 1974*b*).

The bacterial suspension was injected as a single dose into the rumen through the cannula. During administration of the dose the contents of the rumen were thoroughly mixed manually by inserting an arm through the cannula.

Samples from the rumen were taken at various intervals for a period of 9 h, from four different sites. The samples were freed of large particulate matter using specially-built probes covered with fine nylon gauge as described previously (Chaturvedi *et al.* 1973). The rumen fluid samples (35 ml) were transferred to a cold vessel (4°) and were analysed immediately (Singh, Verma *et al.* 1974*b*).

Sampling and analytical procedure for bacterial cells

For both series of experiments the specific radioactivity was expressed as disintegrations/min per mg dry mixed cells. The rumen organisms were withdrawn and analysed, and the radioactivity in the bacterial fraction was determined for the calculations of production rates as described previously (Singh, Verma *et al.* 1974*b*).

RESULTS

The results obtained from different experiments are presented in Tables 1 and 2. Analyses of variance were performed to compare the variations between animal and within animals. It was observed that variations between animals were not different from the variations within animals in the case of DM intake with both the feeding regimens, and for bacteria

Table 1. *Production rates of bacteria and volatile fatty acids* (*VFA*) *in the rumen of buffalo* (Bos bubalis) *calves given green maize*

Animal no.	DM intake (g)	DOM (g)	Isotope used	Dose of isotope (disintegrations/min (×10⁶))	Specific radio-activity at zero time (disintegrations/min per mg dry cells)	Pool size (g)	Turnover time (min)	Bacteria production rate (g/d)	Total VFA (mol/d)
1	3158	2001		17·56	295	59·50	531·36	161·25	18·11
2	2737	1593		18·29	159	57·70	567·36	146·44	16·14
3	3087	1797	¹⁴C-labelled	10·69	204	52·37	453·60	166·24	20·50
1	2543	1484	bacteria	33·20	912	36·40	338·40	154·80	17·45
2	2940	1644		33·20	1000	33·20	324·00	147·40	16·15
3	3335	2024		33·20	955	34·76	305·28	164·00	20·48
1	2248	1509		172·12	4677	36·80	345·60	153·34	17·24
2	3256	1852		140·78	4467	31·51	309·60	146·58	16·13
3	2856	1662		148·59	4169	35·64	292·75	175·31	20·49
1	2110	1232	³⁵S-labelled	115·58	3090	37·40	468·00	115·00	14·00
2	2543	1422	bacteria	115·58	3020	38·27	410·40	134·40	15·49
3	2614	1556		115·58	2884	40·07	396·00	145·00	16·04
1	2199	1284		425·24	10230	39·70	550·08	108·70	12·89
2	2600	1454		425·24	10470	40·61	455·04	128·30	14·28
3	2650	1608		425·24	10470	40·61	432·00	135·40	15·49
Average	2725·06	1608·13						145·477	16·725
SE	165·24	88·65						7·240	1·036

DM, dry matter; DOM, digestible organic matter.

Table 2. *Production rates of bacteria and volatile fatty acids* (*VFA*) *in the rumen of buffalo* (Bos bubalis) *calves given cowpea* (Vigna unguiculata)

Animal no.	DM intake (g)	DOM (g)	Isotope used	Dose of isotope (disintegrations/min (×10⁶))	Specific radio-activity at zero time (disintegrations/min per mg dry cells)	Pool size (g)	Turnover time (min)	Bacteria production rate (g/d)	Total VFA (mol/d)
1	4350	2632		6·58	67·9	96·97	527·04	264·94	21·60
2	3570	2096		6·58	93·3	70·56	440·64	230·58	18·37
3	3625	2073	³⁵S-labelled	85·58	1698·0	50·40	330·76	219·43	16·52
1	4161	2518	bacteria	58·19	660·7	88·08	504·00	251·66	21·60
2	3661	2150		58·19	831·8	69·96	479·52	210·09	16·52
3	3973	2273		6·58	85·5	68·95	422·49	235·02	18·37
1	3919	2663		1·07	12·1	88·50	479·52	265·78	20·60
2	3588	2339	¹⁴C-labelled	1·34	16·2	82·59	505·00	235·52	19·37
3	3315	2107	bacteria	1·34	13·5	99·38	648·00	220·86	17·62
Average	3795·77	2316·77						237·09	18·95
SE	190·023	140·338						11·847	1·169

DM, dry matter; DOM, digestible organic matter.

production in the group given cowpea, and therefore, mean squares based on total obser-
vations were used for the calculation of the standard error. The variations between animals
were significantly greater than variations within animals for bacteria production in green
maize and consequently in such cases values for mean squares between animals were used
to calculate the standard error. The pool size of dry bacterial cells varied from 31·5 to 59·5 g
and 54·4 to 99·4 g in animals given green maize and cowpea respectively. The mean ($\pm$ SE)
production rates of bacterial cells (g/d) and total VFA (mol/d) respectively were
145·477 $\pm$ 7·240 and 16·725 $\pm$ 1·036 with green maize, and 237·090 $\pm$ 11·847 and 18·950 $\pm$ 1·169
with cowpea.

The relationships between bacteria production and the DM intake, DOM and total VFA
produced were derived. Analysis of variance was performed to test the difference between
the individual animals in both slope and displacement of the respective regression lines. It
was observed that the regressions for individual animals did not differ in slope or displace-
ment except in the case of total VFA in the green maize-fed group ($P < 0.05$). Therefore,
the regression equations based on all observations were obtained for all measurements
except total VFA for the green maize-fed group. In the latter case the regression equation
was determined separately for each animal. The bacteria production (Y; g/d) was signi-
ficantly ($P < 0.01$) related to the DM intake (X; g/d). The regression equations of the
relationship were:

for green maize-fed group: $Y = 53.37 + 0.0338X$ (SE $\pm$ 0.0099)

for cowpea-fed group: $Y = 59.83 + 0.0467X$ (SE $\pm$ 0.0146)

DOM was related to the growth of bacteria in the rumen ($P < 0.01$). The relationships
between DOM (x; g/d) and dry bacterial cells (Y; g/d) were as follows:

for green maize-fed group: $Y = 48.1852 + 0.0605x$ (SE $\pm$ 0.0146)

for cowpea-fed group: $Y = 51.7556 + 0.0800x$ (SE $\pm$ 0.0104)

Statistically significant ($P < 0.01$) relationships were also derived between the growth of
rumen bacteria (Y; g/d) and the production of total VFA (X; mol/d) as follows:

for cowpea-fed group: $Y = 58.3748 + 9.4302X$ (SE $\pm$ 1.3369)

for green maize-fed group: animal no. 1: $Y_1 = -29.7383 + 10.5632X$ (SE $\pm$ 0.5074)
animal no. 2: $Y_2 = -20.2235 + 10.2857X$ (SE $\pm$ 1.9013)
animal no. 3: $Y_3 = 44.51312 + 6.0579X$ (SE $\pm$ 1.0632).

DISCUSSION

In ruminants, food proteins are degraded into amino acids by rumen microbes as soon as
they reach the rumen. This is one of the major reasons why simple isotope-dilution tech-
niques could not be applied for measuring the microbial protein synthesis in vivo, because
if labelled protein is used as a marker it may not remain as such after it enters the rumen.
In our earlier studies (Singh, Verma *et al.* 1974a, b), this problem has been avoided by
using labelled microbial cells of rumen origin from animals kept on the same dietary
regimen.

In these experiments the animals were given their ration at 2 h intervals to obtain a steady-
state in the metabolic processes in the rumen. The synthesis of microbial cells would,
therefore, be a continuous process during the period of frequent feeding and the values
could be extrapolated to a 24 h period.

The production rate of bacteria varied from 109 to 265 g/d (Tables 1 and 2). This
variation was due to differences in the foodstuff and the quantities of rations consumed by
the individual animals. There was a linear relationship between the growth of bacteria

in the rumen and the DM intake of the animals. A similar correlation was observed between the DOM and the amount of dry bacterial cells produced daily. In the present experiments the average bacterial cell yield was approximately 107 and 132 g/kg DOM in animals given green maize and cowpea respectively. In previous experiments in which a concentrate ration together with chopped wheat straw was fed to the animals, a bacterial cell yield of 137 g/kg DOM was obtained (Singh, Verma *et al.* 1974*b*). Leng (1972) has proposed a higher yield of rumen bacterial cells, expressed per 100 g DOM, on the basis of theoretical calculations. Most of the stoicheiometric reactions are based on the assumptions that (1) the fermentation is exclusively anaerobic, (2) VFA, carbon dioxide and methane are the only end-products of fermentation, and (3) other hydrogen-consuming reactions such as fatty acid hydrogenation are normally not so important and can be neglected. It is possible that hydrogen, ethanol, lactic acid and formic acid formed in the process of fermentation may be important in some cases and should be taken into account. There is increasing evidence that bacterial cell growth is variable and influenced by the nature of the micro-organisms, availability of energy-yielding substrate (namely carbohydrates), utilizable N compounds or other growth factors in the rumen and the dilution rate (El-Shazly & Hungate, 1965; Hogan & Weston, 1971; Thomas, 1973). The results of many studies using DAPA as a bacterial marker for bacterial cell growth in the rumen have been summarized in a review by Thomas (1973). The cell yield varied from 15·3 to 51·1 g N/kg organic matter fermented. The values are based on different studies using a range of twenty-seven diets. It appears from the results that the extent of protein synthesis is not constant. On the basis of these results the prediction of cell yield, unless supported by direct measurements on similar animals maintained on identical feeding regimens, has been questioned (Annison, 1974). El-Shazly & Hungate (1965), from the 'zero-time rate' method of estimating microbial growth, observed that the net microbial growth rate is usually, if not always, much less than would be possible under ideal growth conditions. The quantity of proteins leaving the rumen may change suddenly on grain-based diets (Ishaque, Thomas & Rook, 1971; Jackson, Rook & Towers, 1971). This change was associated with a large change in the VFA pattern in the rumen, in which there was a change from a high-propionic-producing system to a high-acetate-producing system (Edie & Mann, 1970; Jackson *et al.* 1971). This suggests that the efficiency of synthesis of proteins by the micro-organisms in the rumen varies depending on the composition of the food and microbial community. There appears to be a greater protein synthesis rate in the rumen of sheep on diets that produce a high-propionate fermentation pattern (Hume, 1970).

The bacterial growth in our experiments is much lower than the generally accepted value of 30 g bacteria formed/mol VFA produced. We have observed 76 g bacteria growth/d when 3 mol VFA are produced in goats given Berseem (*Trifolium alexandrinum*) (unpublished observations). In a situation where about 3 to 4 mol VFA are produced in the rumen the value of about 30 g microbes/mol VFA fits in the equation.

In the present studies, linear relationships were observed between the rate of growth of rumen bacterial cells and other measurements e.g. DM intake, DOM and VFA. These studies were based on animals given rations in amounts within a narrow range. It is difficult to extrapolate beyond the experimentally obtained range since it is not certain whether the linear relationship may be obtained in animals with a wide range of intake.

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