DILUTION RATES ON THE EFFICIENCY OF RUMEN MICROBIAL GROWTH IN CONTINUOUS CULTURE

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Introduction

The extent of growth of a microbe is directly proportional to the amount of adenosine triphosphate (ATP) generated from the catabolism of the energy source (Baushop and Elsden, 1960). The value of YATP is varied depending on the specific growth rates and the maintenance energy of microbes. In a continuous culture system, Isaacson et al. (1975) reported that yield glucose increased from 42 to 84 and YATP increased from 7.5 to 16.7, respectively when dilution rate increased from .02 to .12.

Optimum ratio of non protein nitrogen to amino acid nitrogen for microbial growth was 75% urea nitrogen plus 25% amino acid nitrogen (Maeng et al., 1976) and YATP were also improved considerably from 15.4 with 100% urea nitrogen to 20.6 with 75% urea nitrogen +25% amino acid nitrogen (Maeng and Baldwin, 1976).

These studies were initiated to determine the effects of dilution rate, sources of energy and nitrogen on the rumen microbial growth and growth efficiency in a continuous culture system.

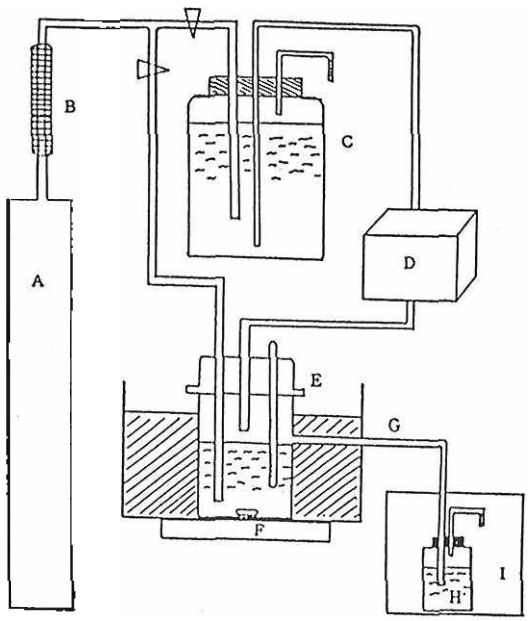
Materials and Methods

Continuous Culture System

Schematic diagram of the continuous culture system is shown in figure 1. Six culture vessels, each 500 ml volume were maintained in water bath heated at 39°C and were agitated continuously with magnetic bar.

Incubation Media

Composition and preparation of culture media were adapted as described by Isaacson et al. (1975). Energy sources were glucose, soluble starch or cellulose and nitrogen sources were 100% urea-N or 75% urea-N plus 25% casein-N.



A. CO_2 gas tank
B. Copper column
C. Media supply bottle
D. Peristaltic pump
E. Fermenter jar
F. Magnetic stirrer
G. Water bath
H. Effluent collection bottle
I. Ice box

Figure 1. Schematic diagram of the chemostat.

Inoculum preparation

Rumen fluid taken from steers was strained through eight layers of cheese cloth and used 250 ml as inoculum.

Dilution rates and sampling

Dilution rates were maintained .02, .05 and .10 h^{-1}. These dilution rates approximate 0.48, 1.20 and 2.40 volume turnover per day. Five days adaptation period were required to achieve steady state condition after which effluent samples were obtained in an ice bath for 3 consecutive days.

Analytical

Cell dry weight was measured as described by Maeng et al. (1976). In cellulose medium cell dry weight was determined by subtraction residual cellulose from dry matter. Glucose was determined by the copperiodometric titration method. Soluble starch was hydrolyzed with 5M H_2SO_4 in a boiling water bath for 4 hour. After neutralization with 5M NaOH, reducing sugar was determined as glucose. Cellulose was determined by the method of Crampton and Maynard (1938). All results were tested by standard analysis of variance procedure and Duncan's multiple range test was used to separate means.

Results and Discussion

Dilution rate and Microbial cell yield

When dilution rates increased from .02 h^{-1} to .10 h^{-1}, microbial dry matter concentration per 100 ml culture medium increased 32.8% with glucose and 9.5% with starch and it decreased 40.5% with cellulose (table 1). Isaacson et al. (1975) also reported that increasing the dilution rate from .02 to .12 h^{-1} with glucose increased the cell concentration by 75%.

Microbial dry matter yields were considerably higher when urea-N replaced with 25% amino acid-N with all energy sources. This is in agreement with other researchers (Maeng and Baldwin, 1976).

Dilution rate and Ysubstrate

Increasing a dilution rate from .02 to .10 h^{-1} increased Ysub by 32.4% with glucose and 8.4% with starch, however it decreased 51.3% with cellulose (table 2). Ysub also increased 42.6% with glucose, 37.1% with starch and 6.1% with cellulose when 100% urea-N replaced with 75% urea-N + 25% amino acid-N.

Ysub values obtained by Bauchop and Elsden (1960) ranged from 8.3 to 23 g of cell dry matter per mole carbohydrate. These values of Bauchop

TABLE 1. EFFECTS OF DILUTION RATES AND NITROGEN SOURCES ON RUMEN MICROBIAL CELL YIELDS*

Item	Dilution rate (h^{-1})			Mean	% increase
	.02	.05	.10		
	mg/100 ml				
Glucose					
100% urea N	57.5±.11[a]	62.5±1.0[b]	71.3±1.1[c]	63.8	100.0
75% urea-N+25% aa-N**	68.8±1.9[a]	93.8±1.3[b]	96.3±1.3[b]	86.3	135.3
Mean	63.1	73.1	83.8		
% increase	100.0	115.9	132.8		
Starch					
100% urea-N	47.4+2.4	49.0±2.2	51.3±3.2	41.2	100.0
75% urea-N+25% aa-N	67.5±2.1[a]	98.8±2.2[b]	107.5±2.3[c]	101.3	245.9
Mean	72.5	73.9	79.4		
% increase	100.0	101.9	109.5		
Cellulose					
100% urea-N	70.9±1.2[b]	47.5±1.5[a]	45.8±1.3[a]	55.1	100.0
75% urea-N+25% aa-N	78.3±1.4[a]	50.8±1.0[b]	43.0±0.5[c]	59.0	107.1
Mean	74.6	49.2	44.4		
% increase	100.0	66.0	59.5		

[a,b,c]Mean ± Standard error and means with different superscript within row differ significantly ($P < 0.05$).
**Acid hydrolyzed casein.

TABLE 2. EFFECTS OF DILUTION RATES AND NITROGEN SOURCES ON THE RUMEN MICROBIAL DM YIELD PER MOLE OF SUBSTRATE*.

Item	Dilution rate (h^{-1})			Mean	% increase
	.02	.05	.10		
	g/mole substrate				
Glucose					
100% urea-N	23.0±2.1	26.0±3.0	28.5±2.2	24.2	100.0
75% urea-N+25% aa-N**	27.5±1.7[a]	37.5±1.2[b]	38.5±2.4[b]	34.5	142.6
Mean	25.3	29.3	33.5		
% increase	100.0	115.8	132.4		
Starch					
100% urea-N	24.9±3.0	23.3±2.1	23.8±2.2	24.0	100.0
75% urea-N+25% aa-N	30.0±1.4[a]	32.9±1.1[ab]	35.8±1.6[b]	32.9	137.1
Mean	27.5	28.1	29.8		
% increase	100.0	102.2	108.4		
Cellulose					
100% urea-N	28.3±2.2[a]	15.8±1.5[b]	15.2±1.9[b]	19.8	100.0
75% urea-N+25% aa-N	31.3±1.8[a]	18.6±1.6[b]	13.7±2.2[b]	21.1	106.1
Mean	29.8	17.2	14.5		
% increase	100.0	57.7	48.7		

*Abbreviations, etc. as in table 1.

and Elsden (1960) and our results were much less than other researchers (Isaacson et al., 1975; Maeng and Baldwin, 1976; Russell and Baldwin, 1979). This may have resulted from differences of ATP yield and the maintenance coefficient and turnover rate due to the different experimental conditions.

Dilution rate and YATP

YATP improved as increasing dilution rate with glucose and starch as an energy source (table 3). At a dilution rate of .02 h^{-1}, YATP was average of 6.4 with glucose and 6.9 with starch. These values increased 7.8 and 8.6 at a dilution rate of .10 h^{-1}. However, with cellulose YATP was 10.8 at a dilution rate of .02 h^{-1} and decreased to 8.5 at dilution rates of .10 h^{-1}. Growth rate of ruminal microbes influences the efficiency of microbial cell yields because it alters the relative proportions of ATP used for maintenance versus growth.

YATP values were also higher when 100% urea-N replaced with 75% urea-N plus 25% amino acid-N with all energy sources. Maeng and Baldwin (1976) also observed that YATP improved from 15.4 with 100% urea-N to 20.6 with 75% urea-N plus 25% amino acid-N.

(**Key Words**: Ysubstrate, YATP, Rumen Microbe)

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TABLE 3. EFFECTS OF DILUTION RATES AND NITROGEN SOURCES ON THE YATP YIELDS*

Item	Dilution rate (h^{-1})			Mean	% increase
	.02	.05	.10		
Glucose					
100% urea-N	6.3±1.0	6.1±2.1	6.8±2.0	6.4	100.0
75% urea-N+25% aa-N**	6.6±1.4	8.8±2.1	8.8±2.2	8.1	126.6
Mean	6.4	7.4	7.8		
% increase	100.0	115.6	121.9		
Starch					
100% urea-N	6.6+1.3	7.2±1.0	8.2±0.8	7.3	100.0
75% urea-N+25% aa-N	7.3±0.1	8.4±0.5	8.9±0.6	8.2	112.3
Mean	6.9	7.8	8.6		
% increase	100.0	113.0	124.6		
Cellulose					
100% urea-N	9.1±2.2	10.4±1.3	9.8±1.4	9.8	100.0
75% urea-N+25% aa-N	12.5±2.6[a]	10.4±1.8[ab]	7.3±2.2[b]	10.1	103.1
Mean	108.0	10.4	8.5		
% increase	100.0	96.3	78.7		

*Abbreviations, etc, as in table 1.
**2.3 mole ATP/mole VFA

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