Influences of Surfactant Tween 80 on the Gas Production, Cellulose Digestion and Enzyme Activities by Mixed Rumen Microorganisms

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ABSTRACT : The surfactant Tween 80 was evaluated for its ability to influence cumulative gas production, cellulose digestion, and enzyme activities by mixed ruminal microorganisms grown on barley grain or Orchardgrass hay. The addition of Tween 80 at a level of 0.10% significantly ($p<0.05$) decreased the cumulative gas production rate from both barley grain or Orchardgrass hay substrates. However, 0.05% Tween 80 did not affect gas production rates compared to the control treatment. The addition of 0.05% Tween 80 to cultures growing on barley grain resulted in a significant increase in cellulase (90.01%), xylanase (90.73%) and amylase (487.25%) activities after 30 h incubation. Cultures utilizing Orchardgrass hay had a significant increase in cellulase (124.43%), xylanase (108.86%) and amylase (271.22%) activities after 72 h incubation. These increases in activities were also observed with cultures supplemented with 0.10% Tween 80 throughout all the incubation times tested. These results indicated that the addition of 0.05% Tween 80 could greatly stimulate the release of some of key enzymes without decreasing cell growth rate in contrast to trends reported with aerobic microorganism. Our data indicates potential uses of the surfactant Tween 80 as a feed additive for ruminant animals. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 8 : 1151-1157)***

Key Words : Tween 80, Rumen Microorganisms, Gas Production, Enzyme Activity, Cellulose Digestion, Feed Additives

INTRODUCTION

Considerable efforts have been devoted to manipulating the rumen environment with the aim of enhancing feedstuff utilization and improving the efficiency of ruminant production. The results of these efforts are a wide range of feed additives that are capable of influencing some component of rumen metabolism. Over the past decade, the manipulations attempted have included methane inhibitors, antibiotics, proteolysis and/or deamination inhibitors, defaunation agents, microbial enzymes, fatty acids and/or lipid feeding, buffer agents and artificial saliva, propionate enhancement by ionophores, probiotics and non-bacterial direct-fed microbials (DFM) including yeast cultures and mold fermentation extracts. The surfactant Tween 80 is well known as an effective surfactant that stimulates the release of enzymes from a range of aerobic fungi (Reese and Maguire, 1969; Schewale and Sadana, 1978; Deshpande et al., 1987; Hung et al., 1988; Yazdi et al., 1990; Long and Knapp, 1991). These experiments were conducted to explore the possibility of using Tween 80 as a feed additive thus alleviating reliance on either antibiotics or ionophores. In previous experiments, we found that this material might be of use as an alternative feed additive that stimulates succinate and lactate utilization by rumen microorganisms in cattle fed high-grain diets, and as an inducer of polysaccharide-degrading enzymes activities. When Tween 80 was included at a concentration of 0.05% (v/v) in the growth medium, this material increased the growth rate of rumen bacteria and fungi, and the rate of cereal grain digestion, succinate and lactate dehydrogenase activities, and polysaccharide-degrading enzymes activities (Lee et al., 2003). The effects of Tween 80 on the cumulative gas production and enzyme activities by mixed rumen microorganisms are reported here.

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MATERIALS AND METHODS

Preparation of ruminal microorganisms

The mixed rumen microorganisms were obtained from a ruminally fistulated Hereford cow fed twice a day (06:00 and 16:00 h), a ration consisting of 70% corn silage, 20% alfalfa hay, 9% soybean meal, trace minerals, and vitamins (13% crude protein diet). Ruminal contents were collected from the bottom of the rumen 4 h after the morning feeding, squeezed through four layers of cheese cloth and poured into a separating funnel that had been gassed with oxygen-free CO_2 and anaerobically incubated at 39°C for up to 60 min to allow feed particles to buoy up. The feed particles that had risen to the surface was removed by a vacuum tube, and then liquid portions were anaerobically filtered using nylon cloth (25 mm pore size) to remove small feed particles. The filtered liquid portions were used as a source of mixed rumen microorganisms.

Culture techniques and media

The anaerobic culture techniques of Hungate (1950) with modifications (Bryant and Burkey, 1953) were used for all incubations. About 75 mg of ground barley grain or

Table 1. Effects of surfactant Tween 80 on cumulative gas production ($ml \cdot g^{-1}$) and its parameters by mixed rumen microorganisms

Incubation time (h)	Treatments		
	Control	0.05%	0.10%
Gas production rate (mL/g DM of substrates) with barley grain			
6	71.17±0.20^{A,a}	79.80±3.47^{a}	7.63±0.67^{b}
12	115.02±1.61^{a}	120.26±3.71^{a}	63.14±1.79^{b}
18	169.89±4.67^{a}	183.87±2.77^{a}	134.26±2.59^{b}
24	208.51±2.74^{b}	220.72±2.36^{a}	173.66±2.55^{c}
30	232.55±1.86^{a}	238.55±2.23^{a}	197.21±2.08^{b}
36	232.55±1.86^{a}	238.55±2.23^{a}	198.93±1.12^{b}
Gas production parameters* with barley grain			
a+b ($mL \cdot g^{-1}$)	221	234	190
k ($Gp \cdot h^{-1}$)	0.0889	0.0943	0.0821
Gas production rate (mL/g DM of substrates) with Orchardgrass hay			
6	26.66±0.88^{a}	28.13±2.26^{a}	7.64±2.37^{b}
12	43.84±1.01	44.26±2.44	29.20±2.88
24	76.95±1.42	72.89±3.03	59.07±2.90
48	134.67±2.33	129.85±3.70	118.16±1.30
72	174.41±3.04	169.68±2.40	161.56±0.77
96	181.26±3.38	177.49±3.03	180.36±1.89
Gas production parameters* with Orchardgrass hay			
a+b ($mL \cdot g^{-1}$)	163	169	201
k ($Gp \cdot h^{-1}$)	0.0382	0.0521	0.0276
lag (h)	7.31	7.33	8.54

^{A}, Mean ± SEM. ^{a,b}, Means with different superscripts in the same rows are different ($p<0.05$).

* The parameters a, b, k and lag for the negative exponential equation. $G_P=a-b[1-\exp^{-k \cdot (time-lag)}]$: G_p is a gas production (mL/1g DM of substrate) of time t: a−b, potential gas production; k, the fractional rate of gas production per hour; lag, time required before rapid gas production began.

Orchardgrass hay (hammer milled with 1mm screen) was added to 30-mL triplicate serum vials (Miller and Wolin, 1974). Anaerobic liquid medium without carbon sources was transferred in 14 mL quantities to each serum vial purged with oxygen-free CO_2. The vials were sterilized by autoclaving at 123°C for 20 min. The pH after autoclaving was 6.65±0.01 and 6.67±0.02 for medium containing with barley grain and Orchardgrass hay, respectively. Incubations were performed anaerobically in batch culture at 39°C without shaking for 0, 6, 12, 18, 24, 30 and 36 h with barley grain and 0, 6, 12, 24, 48, 72 and 96 h with Orchardgrass hay. When Tween 80 was included in the medium, it was added to the medium to yield final concentration of 0.05% (v/v) or 0.10% (v/v) in the medium. The control medium contained no Tween 80. To study the effects of Tween 80 on the growth of ruminal microorganisms, 2.0 mL of ruminal fluid was inoculated into 14 mL of the appropriate medium.

Determination of enzyme activities

Extracellular enzyme activity against carboxymethyl cellulose (CMC) was determined by incubating 0.5 mL of supernatants from microbial cultures (grown under various conditions) with 0.5 ml of 2% (w/v) CMCellulose in 0.1 M Na acetate buffer (pH 5.0). After 2 h incubation, the reaction was stopped by the addition of 0.25 mL of 8% Na_2CO_3. Aliquots were centrifuged at 12,000×g for 5 min, and reducing sugars in the supernatants were assayed colorimetrically by using the DNS (dinitrosalicylic acid) method (Miller, 1959). Xylanase activity and amylase activity were determined by measuring liberated reducing sugar using oat spelts xylan or starch as substrate by the method described above. One international unit (IU) of enzyme activity was defined as the amount of enzyme which liberated 1 mmol of glucose (for CMCase and amylase) and xylose (for xylanase) equivalent per min under the conditions described above.

Gas production determination

At the end of incubation, gas production was determined by using a water displacement apparatus (Fedorak and Hrwdey, 1983). To give a more precise estimate of the gas production throughout the duration of in vitro fermentation (which gave the closest estimate of digestion rates observed in vitro, the following equations were used to analyse the kinetic data by the method described by Herrero et al. (1996) and Merry et al. (1991). In this logistic-like (negative exponential curve) model, the Gp is gas production (mL/g DM of substrate) at time t according to the following equation:

$$GP=a+ b[1-\exp^{-k \cdot (time-lag)}]$$

where, constants a, b, a+b and k are a scale factor (Y-axis intercept), the gas pool size, potential gas production

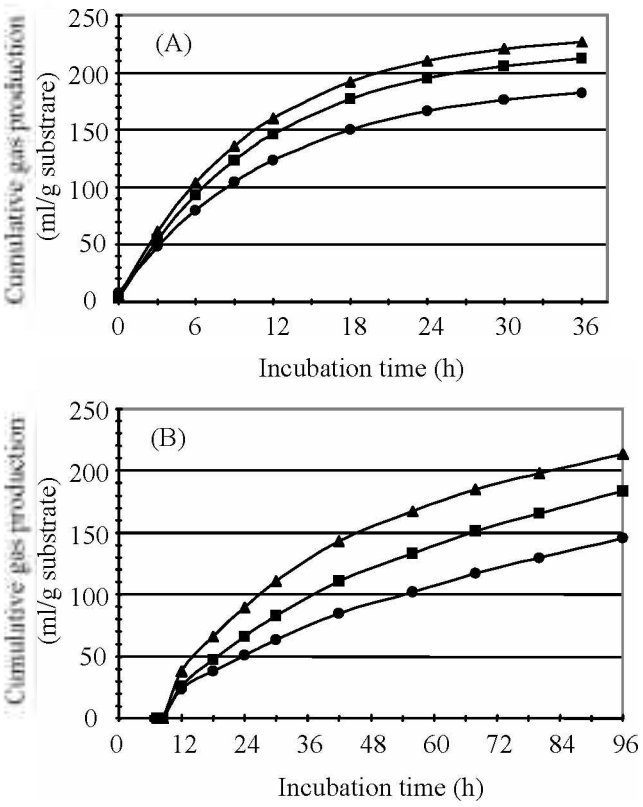

Figure 1. Cumulative gas production profile by rumen microorganisms grown in the medium with added Tween 80 (■-■, Control; ▲-▲, 0.05%; ●-●, 0.10%); representing the rate of gas production expressed in mL gas·g^{-1} DM·h^{-1}. The cumulative gas production profile was derived from a negative exponential equation with lag time: $Gp=a+b[1-exp^{-k\times(time-lag)}]$.

and the rate constant (the fractional rate of gas production per hour), respectively.

The model also allowed for the estimation of a lag phase before rapid gas production began. Gas production and FP cellulose disappearance rate were fitted to a model by the nonlinear (NLIN) procedure of SAS® (1996) using Marquardt's compromise, and then the constants (*a*, *b*, *k* and lag) were evaluated.

Computation of data and statistical analysis

A spread-sheet program (Quatro-Pro, Correll WordPerfect Suite 8) was used for data handling and processing of gas accumulation data. Cumulative gas production data were corrected to 1 g dry matter (DM). NLIN procedure of SAS (1996) was used to fit curves to experimentally derived gas accumulation profiles using the NLIN model of Herrero et al. (1996) and Merry et al. (1991) as described above. Statistical differences were also determined by an analysis of variance with mean separations performed by Duncan's new multiple range test using GLM procedures of SAS (1996), and P value of <0.05 was considered significant.

RESULTS

Gas production

The cumulative in vitro gas production at different times for treatments is given in Table 1. When ruminal microorganisms were incubated with barley, there was no difference in cumulative gas production between the control sample and the sample containing 0.05% Tween 80 but addition of 0.1% Tween 80 significantly ($p<0.05$) reduced the cumulative gas production by 34-63 mL/g at all incubation times compared to the control. The initial rate of gas production in 0.10% Tween 80 group was lower (7.63 ml/g) than those of the control (71.17 ml/g) but with 0.05% Tween 80 there was slightly more gas production (79.80 ml/g). Regardless the treatment, after 30 h of incubation

Table 2. Effects of surfactant Tween 80 on pH of culture supernatant

Incubation time (h)	Treatments		
	Control	0 05%	0.10%
Barley grain			
6	6.20±0.01^{A,b}	6 18±0 00^{b}	6.32±0.02^{a}
12	6.21±0.01^{a}	6 18±0 01^{b}	6.22±0.01^{a}
18	6.26±0.01^{a}	6.20±0.01^{b}	6.14±0.02^{b}
24	6.24±0.01	6.21±0.02	6.18±0.00
30	6.22±0.01^{a}	6 21±0 01^{a}	6.17±0.00^{b}
36	6.19±0.00^{a}	6 15±0 00^{b}	6.16±0.01^{b}
Orchardgrass hay			
6	6.43±0.01	6 51±0 03	6.46±0.02
12	6.41±0.00	6.45±0.00	6.50±0.04
24	6.36±0.00^{c}	6 39±0 00^{b}	6.42±0.01^{a}
48	6.38±0.00	6 40±0 00	6.40±0.03
72	6.42±0.00^{a}	6.41±0.00^{a}	6.37±0.00^{b}
96	6.38±0.00^{a}	6.36±0.01^{ab}	6.34±0.00^{b}

^{A}. Mean ± SEM. ^{a,b}. Means with different superscripts in the same rows are different ($p<0.05$).

cumulative gas production reached the maximum.

In the cultures supplemented with Orchardgrass, the initial rate of gas production was the lowest in the presence of 0.1% Tween 80, but at the end of incubation, there was no difference among the treatments. The control sample and the sample supplemented with 0.05% Tween 80 seemed to reach maximum cumulative gas production after 72 h of incubation, but the addition of 0.1% Tween 80 showed a continual increase in cumulative gas production after 72 h of incubation.

Gas accumulation profiles for each of the treatments and substrates are shown in Figure 1; estimates from fitted curves for parameter values and derived quantities were determined as described in Materials and Methods. In the cultures with barley, there was no lag time before rapid gas production began and there was no difference among the treatments with respect to the rate of production. The addition of 0.05% Tween 80 resulted in the highest and addition of 0.1% Tween 80 resulted in the lowest potential gas production and gas pool size. In the cultures grown with Orchardgrass, the gas production rate showed lag times of 7.31, 7.33 and 8.54 h for the control, 0.05% and 0.10% Tween 80 concentrations, respectively. The gas accumulation rate ($Gp \cdot h^{-1}$) of the control, and 0.05% and 0.10% Tween 80 treatments were 0.04, 0.05 and 0.02, respectively. These results indicated that the addition of 0.05% Tween 80 affected gas pool size in the culture grown with barley and gas accumulation rate in the culture grown with Orchardgrass.

The pH changes in the culture medium of the ruminal microorganisms are shown in Table 2. The initial pH of the culture media decreased slightly after 6 h of growth with both barley and Orchardgrass and there were no differences among the treatments.

Enzyme activity

The time courses enzymatic activities in the different cultures are shown in Table 3, 4 and 5 for CMCase, xylanase and amylase, respectively. CMCase activity in the culture with barley and 0.05% Tween 80 was the highest among the treatments at 12h of incubation through the time courses. The addition of 0.1% Tween 80 showed the highest CMCase activity at 6 h, but activity was dramatically reduced at 12h though this treatment had higher activity than the control at 6 and 18 h. All the treatments showed similar trends in that their activities were the highest at 24 h and decreased at 30 h and increased again at 36 h. In the cultures grown with Orchardgrass, all the treatments showed the same general pattern of increase and decrease of CMCase activities. The addition of 0.05 % Tween 80 resulted in higher activity than the control at 12, 24, 72 and 96 h and the addition of 0.1 % Tween 80 resulted in higher CMCase activity at 24, 48, 72 and 96 h compared to control.

With xylanase activity in the cultures grown with barley, the control had the highest activity at 18 h, the 0.05% Tween 80 treatment had it at 24 h and the 0.1% Tween 80 treatment had highest xylanase activity at 36 h. The control and the culture supplemented with 0.05% Tween 80 showed a decrease in activity after 24 h, but activity in the culture supplemented with 0.1% Tween 80 continuously increased after 12 h and throughout the time courses. The treatment with 0.05% Tween 80 had higher xylanase activity than the control at 12, 18, 24 and 30 h of incubation and the culture supplemented with 0.1% Tween 80 had higher activity than the control at 24, 30 and 36 h. In the cultures grown with Orchardgrass, there were no statistical differences among the treatments except at 24 and 96 h. The control and the culture supplemented with 0.05% Tween 80 showed no increase at 12 h, but by the addition of 0.1% Tween 80, activity continued to increase after 12 h and showed the highest activity among the treatments at 96 h of incubation.

Table 3. Effects of surfactant Tween 80 on CMCase activity ($\mu mol \cdot min^{-1} \cdot ml^{-1}$) in the culture supernatant

Incubation time (h)	Treatments		
	Control	0.05%	0.10%
Barley grain			
6	623.67±60.57^{A,b}	431.35±24.44^{b}	1,494.74±125.96^{a}
12	399.03±30.75	615.59±79.97	313.38±54.92
18	476.60±12.73^{c}	944.46±1.40^{a}	584.88±13.39^{b}
24	563.87±24.19^{b}	1,194.15±22.39^{a}	675.38±6.15^{b}
30	324.69±45.73^{b}	617.20±6.05^{a}	465.29±40.00^{b}
36	369.94±19.53^{b}	854.77±108.87^{a}	596.19±64.70^{ab}
Orchardgrass hay			
6	458.83±39.74	556.48±36.68	471.61±34.46
12	287.52±12.09^{b}	614.64±8.17^{a}	281.42±70.51^{b}
24	625.28±17.31^{c}	1,163.98±31.31^{a}	869.27±49.00^{b}
48	525.09±3.96^{b}	626.43±96.65^{b}	1,372.24±85.41^{a}
72	623.67±16.85^{b}	1,399.75±102.09^{a}	1,586.00±44.73^{a}
96	575.19±18.47^{b}	1,661.45±27.22^{a}	1,534.13±86.63^{a}

^{A}. Mean± SEM. ^{a,b}, Means with different superscripts in the same rows are different ($p<0.05$).

Table 4. Effects of surfactant Tween 80 on xylanase activity (μmol·min^{-1}·ml^{-1}) in the culture supernatant of mixed rumen microorganisms

Incubation time (h)	Treatments		
	Control	0.05%	0.10%
Barley grain			
6	137.05±8.42	162.81± 4.84	126.11± 6.96
12	112.16±9.26^{b}	167.31± 6.69^{a}	78.68± 9.55^{b}
18	137.05±8.20^{b}	256.38±17.60^{a}	130.61± 4.25^{b}
24	117.31±1.82^{c}	266.90±16.74^{a}	181.91±13.08^{b}
30	93.91±6.90^{c}	179.12±15.52^{b}	228.48± 7.29^{a}
36	105.50±3.91^{b}	187.49± 8.26^{ab}	293.51±47.52^{a}
Orchardgrass hay			
6	90.48±4.64	98.21±2.99	91.12± 5.47
12	78.25±2.02	133.40±17.20	86.40± 9.77
24	113.23±5.30^{b}	232.77±23.98^{a}	137.91± 5.29^{b}
48	108.08±3.04	251.02±24.78	222.47±41.48
72	107.43±3.04	260.03±52.29	224.62±74.03
96	110.01±1.40^{b}	229.77±4.38^{ab}	329.57±50.96^{a}

[A] Mean±SEM. [a,b] Means with different superscripts in the same rows are different ($p<0.05$).

Table 5. Effects of surfactant Tween 80 on amylase activity (μmol·min^{-1}·ml^{-1}) in the culture supernatant of mixed rumen microorganisms

Incubation time (h)	Treatments		
	Control	0.05%	0.10%
Barley grain			
6	232.18± 4.98 ^{b}	179.41±10.15 ^{c}	286.38±17.36 ^{a}
12	110.94±13.99 ^{b}	217.21±33.29 ^{a}	103.81±13.16 ^{b}
18	150.17±16.60 ^{c}	453.27± 7.70 ^{a}	229.33± 7.27 ^{b}
24	171.56±18.49	575.94±61.74	411.90±98.45
30	71.72±11.25 ^{b}	421.18±36.38 ^{a}	414.04±50.24 ^{a}
36	94.54± 4.62 ^{c}	429.73±60.13 ^{b}	739.25±99.44 ^{a}
Orchardgrass hay			
6	177.27±25.54	194.06±35.15	130.83±19.40
12	123.78±30.50	188.73±39.19	70.65± 9.66
24	178.69± 2.33	543.73±43.26	441.65±92.67
48	138.04± 7.43 ^{b}	533.07±73.27 ^{a}	677.05±61.42 ^{a}
72	192.24± 9.10 ^{b}	713.62±43.90 ^{a}	851.83±36.35 ^{a}
96	172.99± 1.16 ^{c}	768.47±42.69 ^{b}	1,220.23±86.84 ^{a}

[A] Mean±SEM. [a,b] Means with different superscripts in the same rows are different ($p<0.05$).

Amylase activity in the cultures grown with barley was highest in the control and in the 0.05% Tween 80 treatment after 24 h. but the culture supplemented with 0.1% Tween 80 had highest activity at 36 h. Cultures supplemented with 0.05% Tween 80 had higher amylase activity than the control through the time courses after 6h while the culture supplemented with 0.1% Tween 80 had higher activity at 6, 18. 30 and 36 h. In the cultures grown with Orchardgrass and treated with 0.1% Tween 80 amylase activity continued to increase after 6 h. The addition of 0.05% Tween 80 resulted in higher amylase activity than the control at 24 h and the addition of 0.1% Tween 80 resulted in higher activity at 48 h.

DISCUSSION

Previous experiments on fungal cell permeability demonstrated that non-ionic surfactants (surface active agents) can stimulate the release of enzymes (Reese and Maguire, 1969). Tests conducted on the cellulase complex of the aerobic ascomycetes fungus *Neurospora crassa* showed that the surfactant Tween 80 was effective in stimulating the induction and secretion of enzymes (Yazdi et al.. 1990). Yazdi et al. (1990) have demonstrated that the secretion of several cellulolytic enzymes from *N. crassa* is intimately linked to membrane lipid composition. and the increased release of these enzymes can be explained by effects on membrane fluidity caused by the increased unsaturation of the lipids (Ha et al., 2001: Lee et al.. 2001). The effects of surfactants have been attributed to at least three causes: the decrease in growth rate due to reduced oxygen supply (Hulme and Stranks, 1970), the promotion of the release of bound enzymes (Reese and Maguire. 1969). and action on the cell membrane causing increased

permeability (Reese and Maguire, 1969). If the first effects (decrease of cell growth rate) are detected in anaerobic microorganisms as with aerobic microorganisms, it would be impossible to apply Tween 80 as a feed additive for ruminants. However, the addition of surfactant Tween 80 at the levels of 0.05 and 0.10% did not have any negative effect on the microbial cell growth rate based on gas production rates. There have been many studies to show that growth and fermentation in anaerobic fungi are closely linked. For example, Lowe et al. (1987a,b) showed that the fermentation end-products, formic acid and acetic acid, could be used as a convenient indicator of fungal growth, and Mountfort and Asher (1983) demonstrated that H_2 production could be used as a convenient estimate of fungal growth. Theodorou et al. (1995) demonstrated that measuring the accumulation of fermentation gases during fungal growth is a useful method for the rapid and precise determination of the growth of anaerobic fungi on soluble and particulate substrates. Our results indicated that the addition of Tween 80 did not influence the rate of growth of mixed ruminal organisms. Previous experiments (Lee et al., 2003) in our lab also showed that the growth rates of rumen noncellulolytic bacteria including *Bacteroides amylophilus*, *Megasphaera elsdenii*, *Prevotella ruminicola* and *Selenomonas ruminantium* were greatly increased by the addition of Tween 80 at concentrations of 0.05 and 0.10%. However, growth of cellulolytic bacteria including *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Butyrivibrio fibrisolvens* were only very slightly stimulated or not affected. Growth of rumen mono- and polycentric fungi including *Neocallimastix patriciarum* strain 27 and *Piromyces communis* strain 22, *Orpinomyces joyonii* strain 19-2 and *Anaeromyces mucronatus* strain 543 were greatly improved by the addition of Tween 80 at concentrations of 0.05 and 0.10%.

Previous work (Lee et al., 2002) also showed that cellulase and xylanase activities in the rumen were mostly cell bound and only limited activities were detected in the rumen fluid, or with feed particles. Since these two major cell wall degrading activities were cell associated, it is important to release cell-bound enzymes to the rumen fluid fraction or to the feed particle fraction in order to improve the digestion of feedstuffs.

Although Tween 80 is well known as an effective surfactant in stimulating the release of enzymes from a range of aerobic fungi (Reese and Maguire, 1969; Schewale and Sadana, 1978; Deshpande et al., 1987; Hung et al., 1988; Yazdi et al., 1990; Long and Knapp, 1991), its effects on anaerobic rumen microorganisms have not previously been reported. Our data also showed that the addition of 0.05% Tween 80 to cultures containing barley grain resulted in a significant increase in cellulase (90.01%), xylanase (90.73%) and amylase (487.25%) activities after 30 h incubation. These trends were also observed in cultures containing Orchardgrass hay and supplementation resulted in a significant increase in cellulase (124.43%), xylanase (108.86%) and amylase (271.22%) activities after 72 h incubation. These dramatic increases were observed throughout all the incubation times tested. These results indicate that anaerobic rumen microorganisms are also affected by the addition of Tween 80, and that this surfactant can stimulate the release of some enzymes as has been reported for a range of anaerobic fungi. It is clear from the results presented that Tween 80 can elicit an increase in the amounts of certain cellulolytic enzymes. The effect of Tween 80 may be due to an increase in the permeability of the cell membrane of anaerobic microorganisms, thus permitting more of the enzymes to be secreted, as postulated for other aerobic fungal species by early studies (Demain and Birnbaum, 1968; Reese and Maguire, 1969; Schewale and Sadana, 1978; Yazdi et al., 1990).

In conclusion, the surfactant Tween 80 has a potential for industrial application as a feed additives to improve the digestion of plant material.

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