Effect of Grass Lipids and Long Chain Fatty Acids on Cellulose Digestion by Pure Cultures of Rumen Anaerobic Fungi, *Piromyces rhizinflata* B157 and *Orpinomyces joyonii* SG4

S. S. Lee, J. K. Ha*, K. H. Kim and K. J. Cheng[1]

National Livestock Research Institute, Rural Development Administration, Suweon 441-350, Korea

ABSTRACT : The effects of grass lipids and long chain fatty acids (LCFA; palmitic, stearic and oleic acids), at low concentrations (0.001~0.02%), on the growth and enzyme activity of two strains of anaerobic fungi, monocentric strain Piromyces rhizinflata B157 and polycentric strain Orpinomyces joyonii SG4, were investigated. The addition of grass lipids to the medium significantly ($p<0.05$) decreased filter paper (FP) cellulose digestion, cellulase activity and fungal growth compared to control treatment. However, LCFA did not have any significant inhibitory effects on fungal growth and enzyme activity, which, however, were significantly ($p<0.05$) stimulated by the addition of oleic acid as have been observed in rumen bacteria and protozoa. This is the first report to our knowledge on the effects of LCFA on the rumen anaerobic fungi. Continued work is needed to identify the mode of action of LCFA in different fungal strains and to verify whether these microorganisms have ability to hydrogenate unsaturated fatty acids to saturated fatty acids. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 1 : 23-30*)

Key Words : Long Chain Fatty Acids, Grass Lipids, Rumen Anaerobic Fungus, Cellulose Digestion, Cellulase Activity

INTRODUCTION

Fats and oils are useful energy yielding ingredients in diets for dairy cows to meet the high energy demand for milk production. However, when fats and/or oils are included in ruminant diets, decreases in crude protein, ADF and NDF intakes (Choi and Palmquist, 1996) and their digestibilities (Brooks et al., 1954; White et al., 1958; Czerkawski, 1966; Steele and Moore, 1968) may occur. Long chain fatty acids added to pure cultures of microorganisms also inhibit the growth of certain rumen (Henderson, 1973) and non-rumen (Nieman, 1954) bacteria and protozoa (Ikwuegbu and Sutton, 1982; Hino and Nagatake, 1993), but information with ruminal anaerobic fungi has not been reported.

Anaerobic fungi have been shown to be present in the gut of a wide range of herbivorous animals and the demonstration of the existence of obligate anaerobic fungi has raised many questions concerning their physiology. Much of the evidence for an important contribution by anaerobic fungi to rumen fiber degradation and utilization is based on microscopic observations of rumen digesta and of fungal performance *in vitro* and *in vivo* (Akin et al., 1983; Gordon, 1985; Hillaire et al., 1990). Various workers (Bauchop and Mountfort, 1981; Mountfort et al., 1982; Fonty et al., 1988; Joblin et al., 1990; MarvinSikkema et al., 1990; Joblin and Williams, 1991; Bernalier et al., 1992, 1993) also have shown that anaerobic fungi symbiotically interact with hydrogen-utilizing bacteria, especially methanogens which utilize H_2, a main endproduct produced by fungi in cellulolysis (Wolin and Miller, 1988). Unsaturated fatty acids also have the ability to capture H_2 in the rumen by biohydrogenation (Harfoot, 1978), which reduces the number of double bonds (Ward et al., 1964).

To our knowledge, however, there is little information about the effects of grass lipids and LCFA on the anaerobic fungi. Accordingly we investigated the effects of grass lipids and LCFA on the growth and cellulose digestion by the rumen anaerobic fungi using an anaerobic pure culturing system.

MATERIALS AND METHODS

Fungal strains

The pure strains of anaerobic fungi used were the monocentric strain *Piromyces rhizinflata* B157 isolated from the rumen of bison, and polycentric strain *Orpinomyces joyonii* SG4 isolated from the rumen of sheep. These strains have been characterized in previous publications (Barr et al., 1995). All strains were obtained from the Lethbridge Research Centre Culture Collection (maintenance number: LRC 2265 and LRC 2190 for *P. rhizinflata* B157 and *O. joyonii* SG4, respectively). Fungi were maintained anaerobically in the semidefined liquid medium B of Lowe et al. (1985) containing 1.5 cm^2 of Whatman no. 1

* Address reprint request to J. K. Ha. Department of Animal Science and Technology, Seoul National University, Suweon 441-744, Korea. Tel: +82-331-290-2348, Fax: +82-331-295-7875, E-mail: jongha@snu.ac.kr.

[1] Institute of BioAgricultural Resoures, Academia Sinica, Taipei 115, Taiwan, ROC.

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cellulose filter paper as the sole source of carbohydrate, with subtransfers every three days to maintain viability. A 72 h culture was used as inoculum (10%, v/v) for the triplicate cultures of each time point in the experiment.

Extraction and separation of grass lipids

Fresh alfalfa grass was homogenized in an electric mixer (Brinkmann homogenizer, Model PT 10/35, Brinkmann Instruments Co., Switzerland), and grass lipids were extracted overnight at 4℃ with chloroform-methanol (1:1) by the method of Hino and Nagatake (1993). After filtration, the filtrate was shaken with a half of water and left for 2 h. The chloroform layer was then dehydrated with anhydrous $MgSO_4$ and filtered again. The filtrate was concentrated at room temperature by a rotary evaporator, and used as total grass lipids.

Fatty acids and emulsification

Long chain fatty acids including palmitic acid (hexadecanoic acid, $C_{16:0}$), stearic acid (octadecanoic acid, $C_{18:0}$) and oleic acid (*cis*-9-octadecenoic acid, *cis* $C_{18:1}$) were obtained from Sigma Chemical Co., St. Louis, Mo. They were reported by the manufacturer to be >99.9% pure. Emulsions of grass lipids and fatty acids in autoclaved reducing dH_2O (double distilled water) were prepared by ultrasonication for 4 min using the Vibra Cell™ sonicator (Sonics and Materials Inc., Danbury, Connecticut, USA) set at maximum output under anaerobic condition to prevent oxidation of grass lipids and fatty acids.

Incubation and sampling

To determine the effects of grass lipids and LCFA on the *in vitro* digestion and growth of anaerobic fungi, aliquots of 9.0 mL of medium B (with no added carbohydrates and protein sources) was added under a CO_2 atmosphere to 20 mL culture tubes. Just prior to autoclaving, about 35mg of punched FP as the carbon and energy source were added to each tube. After autoclaving under fast exhaust of steam, emulsified FA were also added at the concentrations of 0.02% (w/v), 0.02% (w/v), 0.01% (w/v) and 0.001% (v/v) for the medium of grass lipids, palmitic acid, stearic acid and oleic acid, respectively. Control medium contained no fatty acid but was added with 0.02% (v/v) of reduced and sterilized distilled water. And tubes were incubated anaerobically (under an initial CO_2 atmosphere) at 39℃. The fungal inocula were composed of 1.0 mL of a 72 h-old culture on filter paper.

Samples were taken at 1, 2, 3, 5 and 7 days of incubation after the inoculation. At each sampling time, three tubes of experimental cultures and two tubes for blank were used. The pH of the medium was measured immediately after the tubes were opened. Liquid fractions were collected for analyses of total protein concentrations and extracellular cellulase activity after centrifugation for 20 min at 3,500 rpm and the residues (FP cellulose and/or attached fungi) were reincubated with pepsin solution to remove fungi from residues. Incubations with pepsin were carried out by the method of Akin (1987) as follows; One hundred milligrams of pepsin (EC3.4.23.1, Sigma) was added to 50 mL of distilled water containing 0.41 mL of concentrated hydrochloric acid. Five milliliters of the solution was added to each tube from which liquid fraction was removed by centrifugation. Tubes were incubated at 25℃ in for 48 h. After incubation, FP was washed under cold tap distilled water using filtering system, and then oven dried for 48 h at 80℃ to allow a measurement of dry matter disappearance. The difference between the initial dry matter weight of the FP and the residual dry matter weight after incubation was taken as FP disappearance. After 3 days of incubation, liquid fraction was also taken before centrifugation for enumeration of fungi using thallus forming units (TFU) method (Theodorou et al., 1990) with five replicates per dilution. Before inoculation of diluted liquid fractions, a filter-sterilized anaerobic solution of streptomycin sulfate, ampicillin sodium salt and chloramphenicol sodium succinate was added to attain a final concentration of 0.1 mg · mL^{-1} of each antibiotic.

Analytical procedures

Anaerobic fungal growth was estimated by TFU and the protein concentration in liquid fraction of culture tubes was assayed with the BioRad (BioRad Laboratories, Richmond, Calif., USA) protein reagent with bovine gammaglobulin as standard. The amount of reducing sugars released in the liquid fraction was determined spectrophotometrically at 600 nm by the method of Miller (1960) using DNS (dinitrosalicylic acid) reagents. Extracellular endoglucanase activity was assayed by the method of Biely et al. (1985) using OBRHEC (ostanzin brilliant redhydroxyethyl cellulose). Endoglucanase activity was expressed against a standard curve of commercial cellulase from *Penicillium funiculosum* (EC3.2.1.4; Sigma Chemical Co., St. Louis, Mo; one unit liberates 1 mole glucose/h from cellulose) and defined as units of commercial cellulase · mL^{-1} h^{-1} incubation.

Statistical analyses

Statistical differences were determined by an analysis of variance with mean separations performed by Duncan's new multiple range test, and p values of <0.05 were considered significant.

Table 1. The protein concentration in the culture medium of anaerobic fungi, *Piromyces rhizinflata* B157 and *Orpinomyces joyonii* SG4 incubated with grass lipids and long-chain fatty acids

Fungal strains	Protein concentration (μmol/mL) after (days);				
Treatments	1	2	3	5	7
***Piromyces rhizinflata* B157**					
Control	11.40±0.57	16.32±0.21^{bc}	25.05±2.11^{bc}	26.30±2.02^{b}	26.47±0.99^{bc}
Grass lipids	10.27±1.03	13.89±0.81^{c}	18.56±3.84^{c}	27.49±1.62^{b}	24.14±1.47^{c}
Palmitic acid	11.01±0.60	12.99±1.13^{c}	27.16±0.56^{bc}	29.15±1.97^{b}	28.95±0.11^{ab}
Stearic acid	12.58±0.55	19.40±1.59^{b}	30.75±2.15^{ab}	32.15±0.89^{ab}	28.69±0.79^{ab}
Oleic acid	12.54±0.83	24.28±0.61^{a}	37.99±1.39^{a}	36.83±0.54^{a}	31.60±0.99^{a}
***Orpinomyces joyonii* SG4**					
Control	8.23±0.63^{b}	10.97±2.36^{b}	33.90±1.71^{b}	29.34±2.02^{b}	27.15±0.70^{b}
Grass lipids	7.72±0.63^{b}	15.24±3.70^{b}	17.49±2.08^{c}	26.67±1.41^{b}	28.16±0.72^{b}
Palmitic acid	10.52±1.26^{ab}	20.55±2.69^{b}	35.25±4.85^{b}	41.62±3.05^{ab}	29.31±2.19^{b}
Stearic acid	13.30±1.48^{a}	32.51±3.89^{a}	45.05±3.08^{b}	45.70±6.06^{a}	34.17±4.69^{b}
Oleic acid	12.18±0.31^{a}	35.09±1.89^{a}	57.68±1.38^{a}	51.05±4.73^{a}	46.25±1.96^{a}

Each value represents the mean±SE of triplicate cultures.
Means in the same column and fungus strain with different superscripts are significantly different ($p<0.05$).

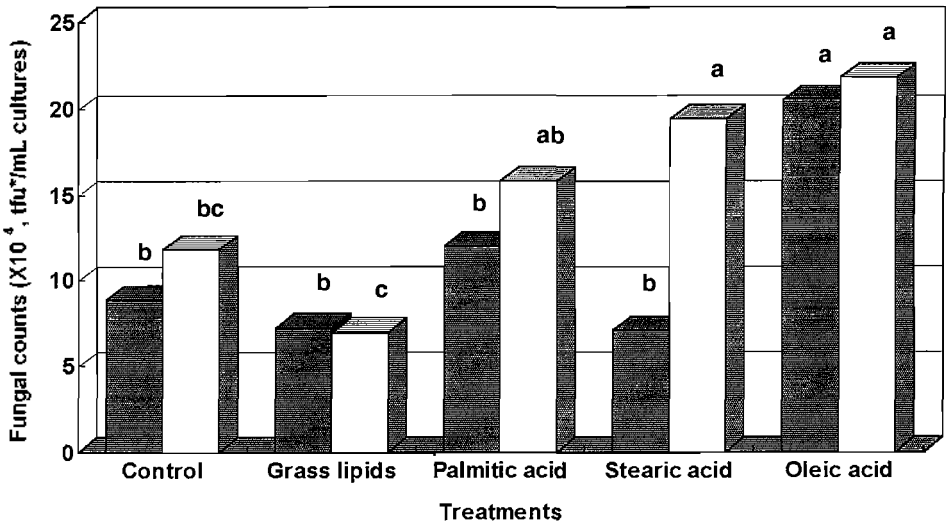

Figure 1. The effects of grass lipids and long chain fatty acids on the fungal counts (*tfu, thallus forming unit) in the culture medium of anaerobic rumen fungi, *Piromyces rhizinflata* B157 (▩) or *Orpinomyces joyonii* SG54 (□). a, b and c different superscripts on the same vertical bars represent significant differences at p=0.05.

RESULTS

Cell growth and protein concentration

The effects of grass lipids and LCFA on the protein concentration as an indicator of cell growth of anaerobic fungal strains are shown in table 1 and those on TFU in rolltubes are presented in figure 1. Although protein concentration in the culture medium was decreased by the addition of grass lipids with statistical differences at two of incubation times, in general there were no significant differences in protein concentration in the supernatant of culture medium in response to the addition of grass lipids and LCFA except oleic acid, which significantly ($p<0.05$) increased protein concentration. The oleic acid stimulated the *in vitro* growth of *P. rhizinflata* B157 and *O. joyonii* SG4, whereas grass lipids inhibited fungal growth as shown in figure 1. Oleic acid induced fungal growth by 2.3 (20.5±4.4) and 1.8 (21.8±1.7) times, while grass lipids inhibited fungal growth by 0.81 (7.2±0.5) and 0.59 (7.0±0.9) times, compared to control treatment for *P. rhizinflata* B157 (8.9±1.2) and *O.*

Table 2. Effect of grass lipids and long-chain fatty acids on filter paper digestion by anaerobic fungi, *Piromyces rhizinflata* B157 and *Orpinomyces joyonii* SG4

Fungal strains	% of dry matter disappearance after (days);				
Treatments	1	2	3	5	7
***Piromyces rhizinflata* B157**					
Control	17.18±0.43	21.05±2.55^{ab}	22.80±4.80^{bc}	86.26±3.81	94.50±0.97^{a}
Grass lipids	15.14±1.38	15.43±0.90^{bc}	17.68±5.61^{c}	86.63±1.94	87.88±2.06^{b}
Palmitic acid	13.22±1.25	13.06±1.54^{c}	30.17±0.62^{bc}	76.27±7.24	96.50±0.37^{a}
Stearic acid	16.55±0.71	21.55±1.77^{ab}	34.17±2.39^{ab}	84.01±3.08	95.63±2.65^{a}
Oleic acid	15.44±1.00	25.03±1.13^{a}	45.51±1.44^{a}	90.64±0.66	97.33±0.40^{a}
***Orpinomyces joyonii* SG4**					
Control	10.60±1.82^{b}	12.19±2.62^{b}	37.67±1.90^{c}	86.51±3.38^{b}	90.51±2.35^{b}
Grass lipids	10.25±1.39^{b}	16.93±4.11^{b}	19.43±2.31^{d}	86.38±0.90^{b}	93.88±2.41^{ab}
Palmitic acid	11.26±0.82^{b}	13.81±3.22^{b}	39.17±5.39^{c}	97.88±0.44^{a}	91.13±1.97^{ab}
Stearic acid	19.19±2.12^{a}	45.41±1.98^{a}	53.28±1.71^{b}	95.25±0.27^{a}	95.88±0.98^{ab}
Oleic acid	17.41±0.45^{a}	38.99±2.10^{a}	66.74±0.67^{a}	96.37±1.23^{a}	98.40±0.43^{a}

Each value represents the mean±SE of triplicate cultures.
Means in the same column and fungus strain with different superscripts are significantly different ($p<0.05$).

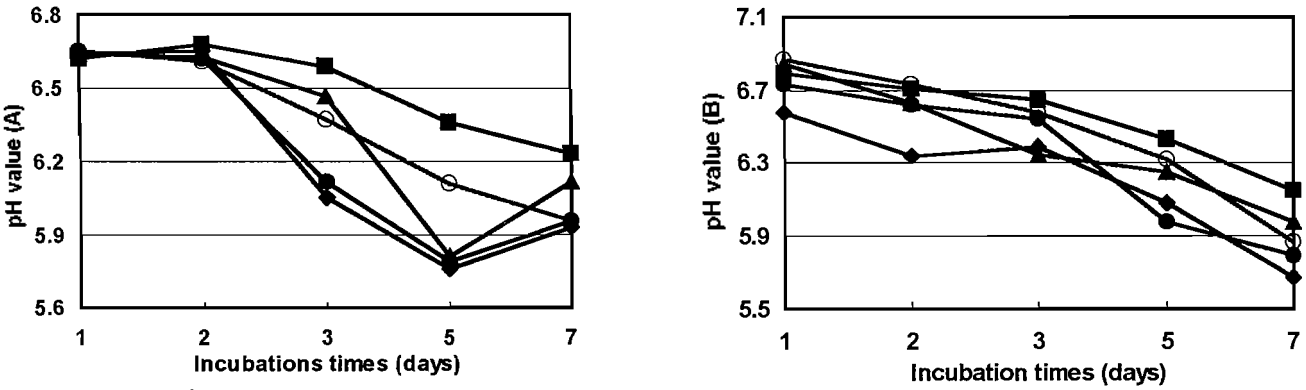

Figure 2. The effects of grass lipids (■—■) and long chain fatty acids (palmitic acid, ▲—▲; stearic acid, ●—●; oleic acid, ◆—◆; and control, ○—○) on the pH value in the culture medium of anaerobic rumen fungi, *Piromyces rhizinflata* B157 (A) or *Orpinomyces joyonii* SG54 (B)

joyonii SG4 ($11.8\pm2.1\times10^{4}$ tfu · mL^{-1}), respectively. In the free acid form, oleic acid increased concentration of protein concentration, and also induced the growth of fungi.

There were few differences in pH among treatments. The initial pH of the culture media was 6.65±0.02. At the 5d of fermentation, the pH of the culture fluid of *P. rhizinflata* B157 with oleic acid was 5.93, whereas the values for the fungal culture of control, grass lipids, palmitic acid and stearic acid were 5.96, 6.23, 6.12 and 5.96, respectively (figure 2). The pH tended to be lower with the addition of oleic acid in the both fungal cultures.

Digestibility and reducing sugar contents

As shown in table 2, the digestibility of FP by *P. rhizinflata* B157 was significantly decreased by the addition of grass lipids, but unaffected by the addition of palmitic and stearic acids, whereas oleic acid markedly enhanced FP digestibility. In the control and grass lipids treatments, the degradation rate by *P. rhizinflata* B157 showed a long lag time of up to 3 days, and a similar trend was also observed when grass lipids was added to *O. joyonii* SG4 culture. However, the degradation rate quickly started to increase from 2 d when incubation medium was added with LCFA. Culture of *O. joyonii* SG4 with oleic acid gave an increased rate of FP cellulose degradation after 2 days incubation, but total FP cellulose degradation among treatments were similar at the end of the fermentation. These results indicate that the degradation rate of FP cellulose by the ruminal fungi

during the early stage of incubation was affected by the addition of grass lipids or LCFA, even though maximum degradation was very similar. However, addition of palmitic acid and grass lipids had a minor effect (not statistically significant) on the digestibility of FP by both species of ruminal fungi, *P. rhizinflata* B157 and *O. joyonii* SG4. This suggests that LCFA do not inhibit the growth or activity of anaerobic fungi as is obserbed in other rumen cellulolytic bacteria and protozoa. *O. joyonii* (37.67%) was slightly more efficient in the degradation of filter paper than P. *rhizinflata* (22.80%) after 72 h incubation, but the difference was not statistically significant.

The concentrations of reducing sugar, expressed as micromoles of glucose per milliliter in the culture medium, began to increase after approximately 3d of incubation, suggesting faster nutrient utilization. However, concentrations of reducing sugar tended to quickly decrease after 5 d of incubation indicating that most of released glucose was fermented soon after liberation from substrate.

Cellulase activity

The endoglucanase activity in the different culture systems by incubation time is presented in table 3. The endoglucanase activity of both of fungal species began to increase from 5 d of incubation, indicating that endoglucanase production by ruminal fungi requires some lag time. The addition of LCFA to the cultures markedly enhanced cellulase activity by anaerobic fungi. The effects of LCFA on FP digestion by fungi correlated closely with their effects on endoglucanase activity. A markedly different type of response was noted with the addition of LCFA. In general, the addition of palmitic acid at the 5 d of incubation showed only mild inhibition on fungal endoglucanase activity, whereas the grass lipids had

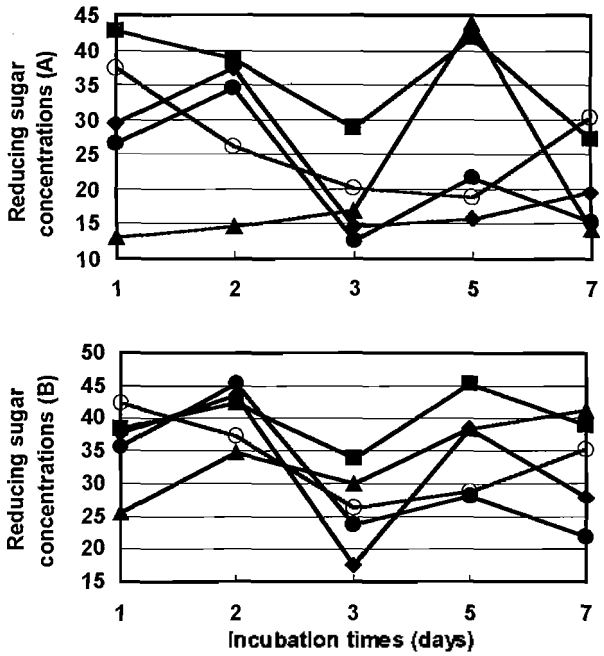

Figure 3. The effects of grass lipids (■—■) and long chain fatty acids (palmitic acid, ▲—▲; stearic acid, ●—●; oleic acid, ◆—◆; and control, ○—○) on the concentrations of reducing sugar (μmol of glucose/mL medium) in the culture medium of anaerobic rumen fungi, *Piromyces rhizinflata* B157 (A) or *Orpinomyces joyonii* SG54 (B)

Table 3. Extracellular OBR-HEC endoglucanase activity in the culture medium of anaerobic fungi, *Piromyces rhizinflata* B157 and *Orpinomyces joyonii* SG4 incubated with grass lipids and long-chain fatty acids

Fungal strains	Cellulase activity (μU/mL/h) after (days);				
Treatments	1	2	3	5	7
***Piromyces rhizinflata* B157**					
Control	0.64 ± 0.02^{c}	0.88 ± 0.03^{b}	1.05 ± 0.11^{a}	8.97 ± 0.06^{a}	8.53 ± 0.11
Grass lipids	0.58 ± 0.01^{c}	0.75 ± 0.01^{c}	1.06 ± 0.11^{a}	5.25 ± 0.56^{c}	8.01 ± 0.26
Palmitic acid	0.74 ± 0.03^{b}	0.85 ± 0.02^{b}	1.06 ± 0.07^{a}	7.10 ± 0.41^{b}	7.96 ± 0.13
Stearic acid	0.83 ± 0.02^{a}	0.89 ± 0.03^{b}	1.13 ± 0.01^{b}	9.15 ± 0.27^{a}	8.38 ± 1.45
Oleic acid	0.86 ± 0.01^{a}	0.98 ± 0.03^{a}	1.19 ± 0.05^{a}	8.84 ± 0.06^{a}	8.97 ± 0.12
***Orpinomyces joyonii* SG4**					
Control	0.88 ± 0.03^{a}	1.01 ± 0.02^{c}	1.44 ± 0.14^{b}	6.81 ± 0.37^{b}	7.27 ± 0.20^{c}
Grass lipids	0.93 ± 0.05^{a}	1.16 ± 0.05^{b}	1.47 ± 0.17^{b}	5.80 ± 0.03^{c}	8.40 ± 0.15^{ab}
Palmitic acid	1.01 ± 0.04^{a}	1.15 ± 0.03^{bc}	1.67 ± 0.20^{ab}	6.66 ± 0.37^{b}	8.70 ± 0.28^{ab}
Stearic acid	0.64 ± 0.07^{b}	1.13 ± 0.04^{bc}	1.59 ± 0.09^{ab}	8.87 ± 0.33^{a}	8.05 ± 0.28^{b}
Oleic acid	0.95 ± 0.12^{a}	1.45 ± 0.08^{a}	1.72 ± 0.11^{a}	8.73 ± 0.09^{a}	8.85 ± 0.12^{a}

Each value represents the meanSE of triplicate cultures.

Means in the same column and fungus strain with different superscripts are significantly different ($p<0.05$).

stronger inhibition. However, stearic or oleic acids did not have appreciable influence on fungal endoglucanase activity.

DISCUSSION

Three decades ago, long chain fatty acids were found to inhibit methane production in the rumen with simultaneous increase in molar proportions of propionic acid (Czerkawski et al., 1966; Demeyer and Hendericks, 1967; Demeyer et al., 1969), showing the possibility of rumen fermentation manipulation with long chain fatty acid. Apart from this aspect, there has been a renewal of interest in the use of high level of lipids and/or fatty acids in the diets of ruminants for the purpose of increasing the energy density of the diet, thus permitting an increased energy intake by high producing lactating cows (Palmquist, 1984). However, when fatty acids and/or oils are included in ruminant diets at a high level, they frequently decrease crude protein, ADF and NDF intakes (Choi and Palmquist, 1996), cellulose, crude fiber, crude protein and nitrogen free extract digestibilities (Brooks et al., 1954; White et al., 1958; Steele and Moore, 1968), methanogenesis (Czerkawski et al., 1966; Prins et al., 1972) and A:P ratio (Jenkins, 1987). Gastrointestinal infusion of long chain fatty acids suppressed dry matter intake in cows (Christensen et al., 1994), and also medium and long chain fatty acids have long been recognized as inhibitors of pure strains of certain rumen (Henderson, 1973) and non-rumen (Nieman, 1954) bacteria and protozoa (Ikwuegbu and Sutton, 1982; Hino and Nagatake, 1993). Although the mechanism of inhibition is not certain, several theories exist to explain the negative effect of lipids and/or fatty acids on cellulose digestibility (Devendra and Lewis, 1974). The speculation that inhibition is due to physical coating of the fiber with lipids is no longer valid, as Ørskov et al. (1978) found no effect on *in sacco* degradability of dried grass coated with tallow. This led the authors to conclude that coating with triglycerides cannot be the reason for the negative effect of LCFA. More recently, Broudiscou et al. (1988) incubated *in sacco* pure cellulose coated with soybean oil hydrolysate (7% w/w) and also found no effect on degradability. A more plausible explanation might be that fatty acids are toxic to certain bacteria and protozoa, and cause shifts in the microbial composition which are responsible for reduced crude fiber digestion. However, there is little information available with ruminal anaerobic fungi which are relatively big contributors to fiber digestion in the rumen. The findings of our study based on cell growth, dry matter disappearance and cellulase activity confirm that cellulolysis by anaerobic fungi was not inhibited by the addition of LCFA, which is different from previously reported results with other rumen microorganisms (bacteria and protozoa). The present study also has shown, for the first time, that cellulolysis by ruminal fungi is markedly stimulated when fungi are grown in medium containing oleic acid. The marked growth stimulation by oleic acid of the anaerobic gut fungal strains is very interesting, because this observation is inconsistent with the theory that the growth of cellulolytic bacteria (*B. flavefaciens*, *R. albus* and *R. flavefaciens*) and protozoa is strongly inhibited by oleic acid. Unexpectedly the addition of LCFA did not inhibit FP digestion. Furthermore, the digestion of FP by anaerobic fungi was also significantly increased by the addition of oleic acid. Presumably, this is due to hydrogenation of UFA, which stimulated the growth and activity of anaerobic fungi by capturing H_2 produced by the fermentation of fungi and/or due to the role of LCFA as growth factors for anaerobic fungi. Hydrogen is an intermediate produced particularly during plant cell wall breakdown by cellulolytic microorganisms, such as *Ruminococcus albus*, *Ruminococcus flavefaciens*, and anaerobic fungi (Stewart and Bryant, 1988; Fonty and Joblin, 1991). Hydrogen never accumulates in the rumen because it is rapidly used by methanogens, which are the dominant hydrogen-utilizing microorganisms in the rumen. Unsaturated fatty acids may also be able to capture hydrogen by saturation of UFA. Continued work is needed to determine whether rumen anaerobic fungi have the ability to hydrogenate UFA to SFA. Another possible explanation is that oleic acid may provide essential fatty acids (Lindsay and Leat, 1977), and thus stimulating ruminal fungi. Orpin and Letcher (1979) showed that growth of *Neocallimastix frontalis* could be stimulated by some unsaturated fatty acids. Oleic acid, present in the rumen fluid in small quantities compared to its 11trans isomers, is the major fatty acid found in all lipids ($C_{16:0}$, 20.7%; $C_{18:0}$, 12.6%; *cis*-$C_{18:1}$, 34.3%) of *N. frontalis* (Body and Bauchop, 1985). The results obtained in this study are, in general, inconsistent with the major effects observed when LCFA and/or oils are added to pure culture of anaerobic bacteria and protozoa. Nonesterified fatty acids are major lipid components of ruminal anaerobic fungi. Apparently, *de novo* synthesis is adequate to meet this demand, or required levels were present in the basal hay substrate. *De novo* synthesis of unsaturated fatty acid in the rumen is questionable (Harfoot, 1978). The reasons for enhanced cellulolysis by oleic acid and stearic acid, but not oil and palmitic acid in fungal cultures are not clear, but may be related to lactate production, which in high concentrations is known to exert a negative effect on cellulolysis. Further work is being carried out to find out mechanisms of stimulation of ruminal fungi by some fatty acids.

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REFERENCES

Akin, D. E. 1987. Use of chitinase to assess ruminal fungi associated with plant residues *in vitro*. Appl. Environ. Microbiol. 53:1955-1958.

Akin, D. E., G. A. R. Gordon and J. P. Hogan. 1983. Rumen bacterial and fungal degradation of *Digitaria pentzii* grown with or without sulfur. Appl. Environ. Microbiol. 46:738-748.

Barr, D. J. S., L. J. Yanke, H. D. Bae, T. A. McAllister and K. J. Cheng. 1995. Contributions on the morphology and taxonomy of some rumen fungi from Canada. Mycotaxon. 54:203-214.

Bauchop, T. and D. O. Mountfort. 1981. Cellulose fermentation by a rumen anaerobic fungus in both the absence and the presence of rumen methanogens. Appl. Environ. Microbiol. 42:1103-1110.

Bernalier, A., G. Fonty, F. Bonnemoy and P. Gouet. 1992. Degradation and fermentation of cellulose by the rumen anaerobic fungi in axenic cultures or in association with cellulolytic bacteria. Curr. Microbiol. 25:143-148.

Bernalier, A., G. Fonty, F. Bonnemoy and P. Gouet. 1993. Inhibition of the cellulolytic activity of *Neocallimastix frontalis* by *Ruminococcus flavefaciens*. J. Gen. Microbiol. 139:873-880.

Biely, P., D. Mislovicova and R. Toman. 1985. Soluble chromogenic substrates for the assay of *endo*-1,4-β glucanase. Anal. Biochem. 144:142-146.

Body, D. R. and T. Bauchop. 1985. Lipid composition of an obligately anaerobic fungus *Neocallimastix frontalis* isolated from bovine rumen. Can. J. Microbiol. 31:463-466.

Brooks, C. C., G. B. Garner, C. W. Gehrke, M. E. Muhrer and W. H. Pfander. 1954. The effect of added fat on the digestion of cellulose and protein by ovine rumen microorganisms. J. Anim. Sci. 13:758-764.

Broudiscou, L., C. J. Van Nevel, D. I. Demeyer and J. P. Jouany. 1988. Addition d'hydrolysate d'huile de soja dans la ration de mouton: effect sur la d gradation *in sacco* de la paille et de la cellulose. Reprod. Nutr. Dëv. 28:159-160.

Choi, B. Y. and D. L. Palmquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. J. Nutr. 126:2913-2919.

Christensen, R. A., J. K. Drackley, D. W. LaCount and J. H. Clark. 1994. Infusion of four longchain fatty acid mixtures into the abomasum of lactating dairy cows. J. Dairy Sci. 77:1052-1069.

Czerkawski, J. W., K. L. Blaxter and F. W. Wainman. 1966. The metabolism of oleic, linoleic and linolenic acids by sheep with reference to their effects on methane production. Br. J. Nutr. 20:349-362.

Demeyer, D. I. and H. K. Henderickx. 1967. The effect of C_{18} unsaturated fatty acids on methane production *in vitro* by mixed rumen bacteria. Biochim. Biophys. Acta. 137:484-497.

Demeyer, D. I., C. J. Van Nevel, H. K. Henderickx and J. A. Martin. 1969. The effect of unsaturated fatty acids upon methane and propionic acid in the rumen. In Energy Metabolism of Farm Animals (Ed. K. L. Blaxter). Oriel Press, Newcastle-upon-Tyne. pp. 139-147.

Devendra, C. and D. Lewis. 1974. The interaction between dietary lipids and fiber in the sheep. Anim. Prod. 19:6771.

Fonty, G. and K. N. Joblin. 1991. The rumen anaerobic fungi. In: Physiological Aspects of Digestion and Metabolism in Ruminants (Ed. T. Tsuda, Y. Sasaki and R. Kawashima). Academic Press, San Diego, California, USA. pp. 625-654.

Fonty, G., E. Grenet, M. Fevre, A. Breton and P. Gouet. 1988. Ecologie et fonction des champignons anaerobies du rumen. Reprod. Nutr. Dev. 28 (Suppl.1):118.

Gordon, G. L. R. 1985. The potential for manipulation of rumen fungi. Rev. Rural Sci. 6:124-128.

Harfoot, C. G. 1978. Lipid metabolism in the rumen. Prog. Lipid Res. 17:21-54.

Henderson, C. 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. 81:107-112.

Hillaire, M. C., J. P. Jouany and G. Fonty. 1990. Wheat straw degradation in Rusitec, in the presence or absence of rumen anaerobic fungi. Proc. Nutr. Soc. 49:127A.

Hino, T. and Y. Nagatake. 1993. The effect of glass lipids on fiber digestion by mixed rumen microorganisms *in vitro*. Jpn. Anim. Sci. Technol. 64:121-128.

Ikwuegbu, O. A. and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr. 48:365-375.

Jenkins, T. C. 1987. Effect of fats and fatty acid combinations on ruminal fermentation in semicontinuous *in vitro* cultures. J. Anim. Sci. 64:1526-1532.

Joblin, K. N. and A. G. Williams. 1991. Effects of cocultivation of ruminal chytrid with *Methanobrevibacter smithii* on lucerne stem degradation and extracellular fungal enzyme activities. Lett. Appl. Microbiol. 12:121-124.

Joblin, K. N., G. E. Naylor and A. G. Williams. 1990. The effect of *Methanobrevibacter smithii* on the xylanolytic activity of rumen fungi. Appl. Environ. Microbiol. 56: 2287-2295.

Lindsay, D. B. and W. M. F. Leat. 1977. Oxidation and metabolism of linoleic acid in fed and fasted sheep. J. Agric. Sci. 89:215-221.

Lowe, S. E., M. K. Theodorou, A. P. J. Trinci and R. B. Hespell. 1985. Growth of anaerobic rumen fungi on defined and semidefined media lacking rumen fluid. J. Gen. Microbiol. 131:2225-2229.

MarvinSikkema, F. D., A. J. Richardson, C. S. Stewart, J. C. Gottschal and R. A. Prins. 1990. Influence of hydrogen consuming bacteria on cellulose degradation by anaerobic fungi. Appl. Environ. Microbiol. 56:3793-3797.

Miller, J. L., R. Blum, W. E. Glennon and A. L. Burton. 1960. Measurement of carboxymethyl cellulase activity. Anal. Biochem. 1:127-132.

Mountfort, D. O., R. A. Asher and T. Bauchop. 1982. Fermentation of cellulose to methane and carbon dioxide by a rumen anaerobic fungus in a triculture with *Methanobrevibacter* sp. strain RA1 and *Methanosarcina barkeri*. Appl. Environ. Microbiol. 44:128-134.

Nieman, C. 1954. Influence of trace amounts of fatty acids on the growth of microorganisms. Bacteriol. Rev. 18:147-163.

Orpin, C. G. and A. J. Letcher. 1979. Utilization of cellulose, starch, xylan and other hemicelluoses for growth by the rumen phycomycete *Neocallimastix frontalis*. Curr. Microbiol. 3:121-124.

Ørskov, E. R., R. S. Hine and D. A. Grubb. 1978. The effect of urea on digestion and voluntary intake by sheep of diets supplemented with fat. Anim. Prod. 27:241-245.

Palmquist, D. L. 1984. Use of fat in diets for lactating dairy cows. In Fat in Animal Nutrition (Ed. J. Wiseman). Butterworths, London, UK. pp. 357-381.

Prins, R. A., C. J. Van Nevel and D. I. Demeyer. 1972. Pure culture studies of inhibitors for methanogenic bacteria. Antonie van Leeuwenhoek. J. Microbiol. 38:281-287.

Steele, W. and J. H. Moore. 1968. The digestibility coefficients of myristic, palmitic acid stearic acids in the diet of sheep. J. Dairy Res. 35:371-376.

Stewart, C. S. and M. P. Bryant. 1988. The rumen bacteria. In The Rumen Microbial Ecosystem (Ed. P. N. Hobson). Elsevier Applied Science, London. pp. 21-75.

Theodorou, M. K., M. Gill, C. KingSpooner and D. E. Beever. 1990. Enumeration of anaerobic chytridiomycetes as thallus forming units: a novel method for the quantitification of fibrolytic fungal populations from the digestive tract ecosystem. Appl. Environ. Micoriol. 56: 1073-1078.

Ward, P. F. V., T. W. Scott and R. M. C. Dawson. 1964. The hydrogenation of unsaturated fatty acids in the ovine digestive tract. Biochem. J. 92:60-68.

White, T. W., R. B. Grainger, F. H. Baker and J. W. Stroud. 1958. Effect of supplemental fat on digestion and the ruminal calcium requirement of sheep. J. Anim. Sci. 17:797-803.

Wolin, M. J. and T. L. Miller. 1988. Microbemicrobe interactions. In: The Rumen Microbial Ecosystem (Ed. P. N. Hobson). Elservier, Amsterdam, The Netherlands. pp. 361-386.