# The Effect of Saturated Fatty Acids on Cellulose Digestion by the Rumen Anaerobic Fungus, *Neocallimatix frontalis* C5-1

**J. K. Ha*, S. S. Lee[1], Z. Gao, C. -H. Kim[1], S. W. Kim, Jong Y. Ko[2] and K. -J. Cheng[3]**
School of Agricultural Biotechnology, Seoul National University, Suweon 441-744, Korea

**ABSTRACT :** The effects of various concentrations of saturated fatty acids (SFA; caprylic, capric and stearic acids) on the growth of the anaerobic fungus, *Neocallimastix frontalis* C5-1 isolated from the rumen of a Korean native goat were investigated. At higher concentrations of fatty acids (0.1%, w/v), the addition of SFA strongly decreased filter paper (FP) cellulose digestion and polysaccharide-degrading enzyme activity. The sensitivity of the rumen anaerobic fungus to the added fatty acids increased in the following order: caprylic ($C_{8:0}$) > capric ($C_{10:0}$) > stearic ($C_{18:0}$) acid, although stearic acid had no significant ($p<0.05$) inhibitory effects at any of the concentrations tested. However, the addition of SFA at lower concentrations (0.01 and 0.001% levels), did not inhibit FP cellulose degradation and enzyme activity. Furthermore, although these parameters were slightly stimulated by the addition of SFA, they were not statistically different from control values. This is the first report examining the effects of fatty acids on anaerobic gut fungi. We found that the lower levels of fatty acids used in this experiment were able to stimulate the growth and specific enzyme activities of rumen anaerobic fungi, whereas the higher levels of fatty acids were inhibitory with respect to fungal cellulolysis. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 7 : 941-946)***

**Key Words :** Fatty Acids, Rumen Anaerobic Fungi, Cellulose Digestion, Cellulase Activity

## INTRODUCTION

The addition of fat to ruminant diets is known to increase the energy density of the diet and milk production. Despite potential improvements in energy intake and milk yield, addition of fat often not only depresses fiber digestibility and methanogenesis in the rumen, but also causes a marked reduction in feed and energy intake when fed to cows in amounts exceeding 50 to 60 g fat/kg dry matter of diet (Schauff and Clark, 1992). Gastrointestinal infusion of long-chain fatty acids has been found to suppress dry matter intake in cows (Christensen et al., 1994), and medium- and long-chain fatty acids have long been recognized as inhibitors of pure strains of microorganisms (Kabara, 1979; Nieman, 1954). Although the mechanism of inhibition is unclear, two theories have been proposed: 1) fatty acids may directly act on ruminal microbes to inhibit their growth and metabolism, or 2) fatty acids may coat fiber particles, thus blocking the action of bacterial cellulases (Devendra and Lewis, 1974). Fatty acids are toxic to bacteria and protozoa in pure culture experiments (Henderson, 1973; Maczulak et al., 1981), but once in the rumen, fatty acids predominately associate with feed particles (Harfoot, 1978). The other possible reason for the observed inhibition (Henderson, 1973) is a decrease in the A/P ratio (Jenkins, 1987), but the degree of inhibition is known to vary with fat and microbial sources. The speculation that inhibition is due to the coating of the fiber with lipids is no longer valid as Ørskov et al. (1978) found no effect on degradability *in sacco* of dried grass coated with tallow. This permitted the authors to conclude that the coating of feed particles with triglycerides cannot account for the observed inhibitory effect. More recently, Broudiscou et al. (1988) incubated *in sacco* pure cellulose coated with soybean oil hydrolysate (7% w/w) and found no effect on degradability. It is now clear that fatty acids themselves are directly toxic to certain bacteria and protozoa. Although anaerobic fungi are recognized as a major component of the cellulose degrading microbiota of ruminants (Phillips and Gordon, 1992), and cellulolysis has a great effect on fatty acids by inhibiting microbial growth, to our knowledge, there is no information regarding the effects of fatty acids on fungal growth or their ability to degrade cellulose. We therefore examined the effects of some of saturated fatty acids on the anaerobic rumen monocentric anaerobic fungi, *Neocallimastix frontalis* C5-1.

* Address reprint request to J. K. Ha. Tel: +82-31-290-2348, Fax: + 82-31-295-7875, E-mail: jongha@snu.ac.kr.
[1] Nutrition Physiology Division, National Livestock Research Institute, RDA, Suweon 441-350, Korea.
[2] Feed & Livistock Research Institute, National Agricultural Co-operative Federation, 424-6 Dang-dong, Goonpo 435-010, Korea.
[3] Institute of BioAgricultural Resoures, Academia Sinica, Taipei 115, Taiwan ROC.
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## MATERIALS AND METHODS

### Fatty acids and dissolution

Commercially available saturated fatty acids (two medium-chains and one long-chain) including caprylic acid (n-octanoic acid, $C_{8:0}$), capric acid (n-decanoic acid, $C_{10:0}$) and stearic acid (octadecanoic acid, $C_{18:0}$) were obtained

from Sigma Chemical Co., (St. Louis, Mo., USA). All were reported by the manufacturer to be approximately >99% pure. These fatty acids were dissolved in absolute ethanol and added to give a final concentration of 0.001, 0.01 and 0.1%, (w/v) in the medium. The final concentration of ethanol in the medium was 0.5% (v/v), which had no effect on the growth or viability of the fungus. In preliminary experiments (data not shown), we found that addition of up to 0.5% (v/v) ethanol alone had no effect on the pH, cellulose degradation rate, enzyme activity or growth rate of *N .frontalis* C5-1, although inhibition was observed when the greater amounts of ethanol (2.5% and 5%) were added.

**Fungal strains and culture conditions**

A pure strain of *N. frontalis* strain C5-1, which is characterized by a monocentric thallus and polyflagellated zoospores (Orpin, 1975), was isolated in our laboratory from the ruminal contents of a Korean native goat (Ha et al., unpublished data). Fungal cultures were prepared and grown under strict anaerobiosis according to the methods of Hungate (1950) as modified by Bryant and Burkey (1953). The fungus was maintained anaerobically on a basal medium containing cellulose or cellobiose (0.2%, w/v) as the sole carbon and energy source (pH=6.8), with subtransfers every three days to maintain viability. The basal medium, which was modified from that of Ho et al. (1988), consisted of salts solution A (16.5 ml), salts solution B (16.5 ml), clarified rumen fluid (17.0 ml), yeast extract (100 mg; Sigma Chemical Co., St. Louis, Mo., USA), trypticase peptone (100 mg; Bection Dickinson and Company, Cockeysville, MD, USA), 0.1% (w/v) resazurin (1.0 ml; Sigma Chemical Co., St. Louis, Mo., USA), pre-reduced distilled water (50.0 ml), $NaHCO_3$ (500 mg; Sigma Chemical Co., St. Louis, Mo., USA), and cystein hydrochloride (100 mg; Sigma Chemical Co., St. Louis, Mo., USA). Salts solution A and B were those described by Hungate (1969). For the preparation of experimental cellulose medium, pieces of Whatman no. 1 filter paper (approximately 50 mg; Whatman International Ltd, Kent, UK) were placed in the bottom of each tube and pre-reduced basal medium (9 ml) was poured into each tube. The basal medium was dispensed under an atmosphere of oxygen-free $CO_2$ into Pyrex® (No. 9820) culture tubes that had been pre-filled with oxygen-free $CO_2$. The tubes were then plugged with butyl rubber stoppers and autoclaved under fast exhaust. After autoclaving, antibiotic mixtures, fatty acids and fungal inoculum were added to each tube. An antibiotic mixture was added (0.2 ml of 2% (v/v) stock) as a purely precautionary measure to prevent bacterial contamination. This mixture contained 1.212% (w/v) benzylpenicillin, 0.265% (w/v) streptomycin sulphate and 0.060% (w/v) chloramphenicol. The antibiotics were dissolved in distilled water that had been freshly boiled and cooled under an atmosphere of $CO_2$, and were dispensed anaerobically into serum bottles, which were stoppered and sealed. The fungal inoculum consisted of a 1.0 ml sample (10% of the volume) of a 72 h culture supernatant grown in the basal medium. All incubations were carried out at 39°C in an upright position without shaking.

**Sampling and analysis**

FP cellulose degradation and enzyme activities were determined in triplicate for each treatment. Cultures were harvested after 0, 2, 4 and 6 d of incubation. Supernatants were separated from the residual particulate substrate and adherent fungal biomass by centrifugation at 3,000 rpm for 20 min. Supernatants from three replicate cultures were analyzed for enzyme activities and each measurement was performed in triplicate. A volume of 0.5 ml of the supernatant (crude enzyme solution) was mixed with 0.5 ml of a 1% CMC (carboxymethylcellulose; Sigma Chemical Co., St. Louis, Mo., USA) solution in 0.05 M citrate buffer (pH 5.5). The reaction was incubated for 1 h at 55°C (without shaking), samples were boiled for 5 min to stop the reaction, followed by centrifuged at 7,000 rpm for 5 min. Reducing sugar released into the supernatant was measured colorimetrically using the DNS (dinitrosalicylic acid; Sigma Chemical Co., St. Louis, Mo., USA) method of Miller et al. (1960). One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of glucose or xylose equivalent of reducing sugar per minute under the above conditions. Xylanase activity was assayed with 1.0 mL of 2% oat spelts xylan (w/v) in 0.5 M potassium phosphate buffer (pH 6.5). The liberated reducing sugar was assayed as described above. To determine the extent of FP cellulose degradation, samples were boiled in 1 M NaOH (to remove adherent fungal matter), rinsed three times with absolute alcohol (60°C), twice with running distilled water, and dried until a constant weight was achieved (approximately 12 h at 78°C). The FP cellulose degradation was expressed as a percentage (mean±SE) of that in control fungal cultures. For measurement of volatile fatty acids, a 1.0 ml portion of the supernatant was acidified with 0.2 ml phosphoric acid at room temperature for 30 min. The sample was centrifuged at 3,000 rpm for 10 min, and the VFA (except formic acid) were determined by gas chromatography.

**Statistical analysis**

Statistical differences were determined by an analysis of variance with mean separations performed by Duncan's multiple range test using GLM (general linear model) procedures of SAS (1989).

## RESULTS

**Effects of fatty acids on cellulose degradation**

The effects of various levels of fatty acids on the FP

**Table 1.** Effects of saturated fatty acids on the degradation rate (%) of filter paper cellulose by ruminal anaerobic fungus, *Neocallimastix frontalis* C5-1

| Treatments | Incubation Time (days) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Control | 0.61 ± 0.50 | $10.32^{abc}$ ±0.31 | $58.91^{a}$ ± 4.62 | $82.78^{a}$ ±0.93 |
| Caprylic acid | | | | |
| 0.1% | 0.00 ±0.00 | $0.00^{d}$ ±0.00 | $0.0^{b}$ ± 0.00 | $0.98^{e}$ ±0.80 |
| 0.01% | 0.00 ±0.00 | $1.62^{d}$ ±0.66 | $44.34^{a}$ ±8.78 | $48.27^{c}$ ±9.02 |
| 0.001% | 0.00 ±0.00 | $12.95^{ab}$ ±2.18 | $58.94^{a}$ ±3.83 | $81.38^{a}$ ±2.00 |
| Capric acid | | | | |
| 0.1% | 0.00 ±0.00 | $1.22^{d}$ ±1.00 | $13.16^{b}$ ±3.31 | $28.09^{d}$ ±3.31 |
| 0.01% | 0.77 ±0.63 | $14.67^{a}$ ±1.99 | $71.05^{a}$ ±1.56 | $80.32^{ab}$ ±1.29 |
| 0.001% | 0.96 ±0.78 | $16.71^{a}$ ±3.51 | $69.41^{a}$ ±1.91 | $80.38^{ab}$ ±3.56 |
| Strearic acid | | | | |
| 0.1% | 0.00 ±0.00 | $3.83^{cd}$ ±3.13 | $43.36^{a}$ ±11.63 | $63.25^{bc}$ ±8.03 |
| 0.01% | 0.00 ±0.00 | $6.04^{bcd}$ ±2.49 | $56.18^{a}$ ±3.17 | $79.34^{ab}$ ±1.65 |
| 0.001% | 0.00 ±0.00 | $6.03^{bcd}$ ±1.15 | $59.07^{a}$ ±5.67 | $69.72^{ab}$ ±4.33 |

Each value represents the mean±SE of triplicate cultures.
$^{a,b,c,d}$ Means in the same column with different superscripts differ significantly ($p<0.05$).

**Table 2.** Effects of saturated fatty acids on the supernatnat pH of cultures of the ruminal anaeobic fungus, *Neocallimastix frontalis* C5-1

| Treatments | Incubation Time (days) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Control | $6.67^{de}$ ±0.00 | $6.55^{e}$ ±0.01 | $5.76^{f}$ ±0.01 | $5.70^{f}$ ±0.01 |
| Caprylic acid | | | | |
| 0.1% | $6.68^{cd}$ ±0.00 | $6.71^{a}$ ±0.00 | $6.67^{a}$ ±0.00 | $6.67^{a}$ ±0.00 |
| 0.01% | $6.68^{cd}$ ±0.00 | $6.67^{b}$ ±0.00 | $6.42^{c}$ ±0.01 | $6.41^{c}$ ±0.01 |
| 0.001% | $6.69^{bc}$ ±0.00 | $6.67^{b}$ ±0.00 | $6.04^{e}$ ±0.02 | $5.95^{e}$ ±0.01 |
| Capric acid | | | | |
| 0.1% | $6.66^{e}$ ±0.00 | $6.58^{d}$ ±0.00 | $6.57^{b}$ ±0.01 | $6.56^{b}$ ±0.01 |
| 0.01% | $6.70^{b}$ ±0.01 | $6.57^{de}$ ±0.01 | $6.58^{b}$ ±0.00 | $5.94^{e}$ ±0.00 |
| 0.001% | $6.69^{cd}$ ±0.00 | $6.56^{de}$ ±0.01 | $6.04^{e}$ ±0.01 | $5.93^{e}$ ±0.02 |
| Stearic acid | | | | |
| 0.1% | $6.73^{a}$ ±0.01 | $6.66^{bc}$ ±0.01 | $6.14^{d}$ ±0.04 | $6.31^{d}$ ±0.01 |
| 0.01% | $6.74^{a}$ ±0.00 | $6.64^{c}$ ±0.00 | $6.15^{d}$ ±0.02 | $5.88^{e}$ ±0.01 |
| 0.001% | $6.69^{bc}$ ±0.00 | $6.66^{b}$ ±0.00 | $6.19^{d}$ ±0.03 | $5.86^{e}$ ±0.06 |

Each value represents the mean±SE of triplicate cultures.
$^{a,b,c,d,e,f}$ Means in the same column with different superscripts differ significantly ($p<0.05$).

cellulose degradation by *N. frontalis* C5-1 are shown in table 1. The rate of FP degradation was significantly ($p<0.05$) inhibited by the addition of medium-chain fatty acids at a concentration of 0.1%, but not by any other concentration. The inhibitory effects of the medium-chain fatty acids (caprylic and capric acids) were greater than those of the long-chain fatty acid (stearic acid). Between the medium-chain fatty acids, caprylic acid inhibited against cellulose degradation by *N. frontalis* C5-1 to a greater extent than capric acid. Stearic acid had no significant inhibitory effects at any concentration, or incubation time tested, except after the 6 d incubation with 0.1% stearic acid. The shorter chain fatty acids were more inhibitory, possibly as a result of their greater solubility. Inhibition by fatty acids was never observed when the fatty acid was present at level of 0.001%. The FP cellulose degradation rate was inversely proportional to the fatty acid concentration added. The highest concentration of capric acid (0.1%) inhibited cellulose degradation by the fungus, whereas the lower concentrations slightly stimulated cellulose degradation, although the stimulation was not statistically significant ($p>0.05$).

The pH changes in cultures of the fungus are shown in table 2. The pH was found to vary with treatments and incubation times. Generally, the initial pH of the culture media (6.69±0.01) declined during the incubation period. The pH of control culture was the lowest ($p<0.05$) at all incubation times, and the culture supplemented with long-chain fatty acid was lower in pH than those supplemented with the medium-chain fatty acids after 4 and 6 d.

**Effects of fatty acids on VFA production**

The presence of saturated fatty acids greatly affected the production of total VFA and acetic acid (table 3). The total VFA production by the fungus was slightly inhibited by the addition of the highest concentrations of fatty acids tested, although these values were not statistically different from the control treatment. However, the acetic acid production was significantly ($p<0.05$) inhibited by the addition of fatty acids at a concentration of 0.1%, but not at any other concentrations. In contrast, the ratio of acetate to total VFA tended to increase at the lower concentrations, although this was not statistically different from the control treatment.

**Effects of fatty acids on enzymatic activity**

The time courses of the enzymatic activities monitored in the culture supernatants are shown in tables 4 and 5. The

**Table 3.** Effects of saturated fatty acids on the changes of VFA concentrations (mmol/l) in the culture medium incubated for 6 days with ruminal anaeobic fungus, *Neocallimastix frontalis* C5-1

| Treatment | Total VFA | Acetate | Acetate/ Total VFA |
|---|---|---|---|
| Control | 40.05±3.59 | 23.79$^{a}$ ±0.49 | 0.61$^{abc}$ ±0.07 |
| Caprylic acid | | | |
| 0.1% | 25.92±1.09 | 11.02$^{c}$ ±0.22 | 0.43$^{c}$ ±0.03 |
| 0.01% | 31.06±3.56 | 18.93$^{ab}$ ±1.51 | 0.63$^{ab}$ ±0.08 |
| 0.001% | 38.17±2.38 | 24.78$^{a}$ ±1.33 | 0.65$^{ab}$ ±0.02 |
| Capric acid | | | |
| 0.1% | 24.92±3.47 | 14.40$^{bc}$ ±0.65 | 0.60$^{abc}$ ±0.06 |
| 0.01% | 24.83±3.48 | 19.46$^{ab}$ ±2.73 | 0.79$^{a}$ ±0.03 |
| 0.001% | 33.68±5.87 | 23.88$^{a}$ ±3.17 | 0.73$^{a}$ ±0.04 |
| Stearic acid | | | |
| 0.1% | 18.73±3.64 | 9.90$^{c}$ ±0.38 | 0.46$^{bc}$ ±0.04 |
| 0.01% | 32.56±0.30 | 20.45$^{ab}$ ±1.17 | 0.63$^{ab}$ ±0.04 |
| 0.001% | 30.64±4.21 | 19.66$^{ab}$ ±3.18 | 0.64$^{ab}$ ±0.03 |

Each value represents the mean±SE of triplicate cultures.
$^{a,b,c,d,e}$ Means in the same column with different superscripts differ significantly ($p<0.05$).

CMCase activity of the control treatment increased rapidly throughout duration of incubation time. Addition of all tested fatty acids at the 0.1% concentration, and caprylic acid at the 0.01% concentration, caused almost complete inhibition of the CMCase activity in the supernatant of pure culture of *N. frontalis* C5-1. The CMCase activity was not detected in culture containing 0.1% caprylic acid. Among the tested fatty acids, caprylic acid was the most potent inhibitor of the fungal CMCase activity when *N. frontalis* C5-1 was supplemented with 0.1% and 0.01% caprylic acid. Inhibition was dose dependent as inhibition of the CMCase activity by capric and stearic acids were not observed when these fatty acids were present as low as 0.01%. Furthermore, the addition of capric and stearic acids at lower concentrations (0.01 and 0.001%) had a stimulatory effect (not statistically significant) on the fungal CMCase activity. The results suggest that lower levels of fatty acids do not inhibit the growth rate or enzymatic activities of anaerobic fungi inversely, as has been reported for other ruminal microorganisms such as cellulolytic bacteria and protozoa (Nieman, 1954).

As was seen in the rate of cellulose degradation and CMCase activity, the fungal xylanase activity was significantly reduced by the addition of 0.1% caprylic acid, but not by the lower concentrations (table 5). In contrast to its effects on FP cellulose degradation and CMCase activity, 0.1% capric acid did not significantly inhibit the fungal xylanase activity ($p>0.05$) except after 2 d incubation with 0.1% capric acid or 4 d incubation with 0.01 and 0.001% capric acid. Stearic acid (all concentrations tested) did not inhibit the fungal xylanase activity after 2 d, but significant ($p<0.05$) inhibition was observed after 4 d incubation. This inhibition was completely overcome by 6 d incubation.

**Table 4.** Effects of saturated fatty acids on the CMCase activity ($\mu$mol·glucose·min$^{-1}$·ml$^{-1}$) in the supernatant of culture medium of ruminal anaeobic fungus, *Neocallimastix frontalis* C5-1

| Treatments | Incubation Time (days) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Control | 0.00 ±0.00 | 160.00$^{bc}$ ±12.50 | 447.67$^{b}$ ±42.45 | 516.00$^{a}$ ±33.33 |
| Caprylic acid | | | | |
| 0.1% | 0.00 ±0.00 | 0.00$^{f}$ ±0.00 | 9.33$^{e}$ ±3.14 | 0.00$^{c}$ ±0.00 |
| 0.01% | 0.00 ±0.00 | 57.67$^{de}$ ±2.13 | 319.00$^{c}$ ±64.03 | 150.00$^{bc}$ ±1.73 |
| 0.001% | 0.00 ±0.00 | 149.67$^{bc}$ ±8.38 | 461.33$^{ab}$ ±17.83 | 547.00$^{a}$ ±34.32 |
| Capric acid | | | | |
| 0.1% | 0.00 ±0.00 | 41.67$^{e}$ ±10.86 | 143.33$^{d}$ ±31.82 | 118.67$^{c}$ ±17.48 |
| 0.01% | 0.00 ±0.00 | 179.00$^{ab}$ ±15.20 | 579.33$^{a}$ ±28.77 | 567.00$^{a}$ ±15.12 |
| 0.001% | 0.00 ±0.00 | 209.67$^{a}$ ±12.93 | 552.00$^{ab}$ ±16.44 | 556.00$^{a}$ ±19.29 |
| Stearic acid | | | | |
| 0.1% | 0.00 ±0.00 | 47.33$^{de}$ ±10.00 | 282.67$^{c}$ ±7.66 | 295.00$^{b}$ ±104.31 |
| 0.01% | 0.00 ±0.00 | 132.00$^{c}$ ±4.50 | 527.33$^{ab}$ ±20.13 | 562.67$^{a}$ ±39.31 |
| 0.001% | 0.00 ±0.00 | 83.67$^{d}$ ±13.55 | 509.00$^{ab}$ ±14.29 | 466.33$^{a}$ ±69.74 |

Each value represents the mean±SE of triplicate cultures.
$^{a,b,c,d,e}$ Means in the same column with different superscripts differ significantly ($p<0.05$).

## DISCUSSION

Of the series of saturated fatty acids, the shorter-chain caprylic acid ($C_{8:0}$) had a greater inhibitory effect on *N. frontalis* C5-1 than the longer-chain stearic acid ($C_{18:0}$) even when present at the highest concentration (table 6). The decrease in inhibition with increasing chain length may be related to the fact that the longer-chain fatty acids are more hydrophobic and thus, have a lower solubility. These fatty acids are therefore less likely to be absorbed onto feed particles or onto microbial cells. Inhibitory fatty acids must be sufficiently water soluble to reach an effective concentration in the aqueous solution and yet sufficiently hydrophobic to interact with the hydrophobic proteins and lipids on the fungal cell surface. Stearic acid is virtually insoluble in water. We also observed (optically) that stearic acid that had been dissolved in absolute ethanol recoagulated in the fungal cultures. However, relevant information on solubility of fatty acids in rumen liquid or *in vitro* medium is unavailable. Previous research has shown that there is an inverse relationship between the melting points of lipid and the digestibility coefficients of fatty acids (Carroll, 1958; Cheng et al., 1949) and that the fatty acid melting point and solubility are positively correlated

**Table 5.** Effects of saturated fatty acids on the xylanase activity (molxylosemin$^{-1}$ml$^{-1}$) in the supernatant of culture medium of ruminal anaeobic fungus, *Neocallimastix frontalis* C5-1

| Treatments | Incubation Time (days) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Control | 82.33$^{b}$ ±2.76 | 424.33$^{a}$ ±12.30 | 586.33$^{a}$ ±49.28 | 517.67$^{a}$ ±71.12 |
| Caprylic acid | | | | |
| 0.1% | 86.67$^{ab}$ ±0.98 | 88.3$^{c}$ ±1.19 | 112.33$^{e}$ ±7.22 | 80.67$^{b}$ ±2.42 |
| 0.01% | 92.33$^{a}$ ±5.82 | 331.00$^{b}$ ±9.81 | 475.00$^{bcd}$ ±10.87 | 439.00$^{a}$ ±8.66 |
| 0.001% | 90.33$^{ab}$ ±0.27 | 394.33$^{a}$ ±3.57 | 508.00$^{abc}$ ±15.12 | 491.33$^{a}$ ±78.17 |
| Capric acid | | | | |
| 0.1% | 95.67$^{a}$ ±0.98 | 291.33$^{b}$ ±26.65 | 524.67$^{ab}$ ±34.51 | 533.67$^{a}$ ±29.29 |
| 0.01% | 90.67$^{ab}$ ±2.68 | 401.00$^{a}$ ±15.33 | 421.67$^{cd}$ ±18.68 | 345.00$^{a}$ ±12.12 |
| 0.001% | 87.33$^{ab}$ ±1.52 | 441.67$^{a}$ ±8.46 | 450.33$^{bcd}$ ±14.56 | 383.00$^{a}$ ±8.96 |
| Stearic acid | | | | |
| 0.1% | 86.67$^{ab}$ ±1.19 | 424.33$^{a}$ ±14.31 | 428.67$^{cd}$ ± 0.98 | 433.33$^{a}$ ±13.53 |
| 0.01% | 87.33$^{ab}$ ±0.27 | 433.33$^{a}$ ±2.60 | 417.33$^{d}$ ±14.43 | 568.67$^{a}$ ±118.94 |
| 0.001% | 95.00$^{a}$ ±2.05 | 443.00$^{a}$ ±7.87 | 460.67$^{bcd}$ ±9.22 | 484.33$^{a}$ ±24.07 |

Each value represents the mean±SE of triplicate cultures.
$^{a,b,c,d}$ Means in the same column with different superscripts differ significantly ($p<0.05$).

**Table 6.** Relative values (%)[1] of DMD, CMCase and xylanase activity after 4 days incubation with fungal cultures containing different levels of saturated fatty acids

| Treatments | DMD | CMCase | Xylanase |
|---|---|---|---|
| Control | 100.00$^{ab}$ ± 7.95 | 100.00$^{b}$ ±9.48 | 100.00$^{a}$ ±8.41 |
| Caprylic acid | | | |
| 0.1% | 0.00$^{c}$ ± 0.00 | 2.09$^{e}$ ±0.70 | 19.16$^{e}$ ±1.23 |
| 0.01% | 75.26$^{b}$ ±15.12 | 71.26$^{c}$ ±14.30 | 81.01$^{bcd}$ ±1.85 |
| 0.001% | 100.06$^{ab}$ ± 6.60 | 103.05$^{ab}$ ±3.98 | 86.64$^{abc}$ ±2.58 |
| Capric acid | | | |
| 0.1% | 22.34$^{c}$ ±5.69 | 32.02$^{d}$ ±7.11 | 89.49$^{ab}$ ±5.89 |
| 0.01% | 120.62$^{a}$ ±2.69 | 129.41$^{a}$ ±6.43 | 71.92$^{cd}$ ±3.19 |
| 0.001% | 117.82$^{a}$ ±3.29 | 123.31$^{ab}$ ±3.67 | 76.80$^{bcd}$ ±2.48 |
| Stearic acid | | | |
| 0.1% | 73.61$^{b}$ ±20.01 | 63.14$^{c}$ ±1.71 | 73.11$^{cd}$ ±0.17 |
| 0.01% | 95.36$^{ab}$ ±5.45 | 117.79$^{ab}$ ±4.50 | 71.18$^{d}$ ±2.46 |
| 0.001% | 100.27$^{ab}$ ±9.77 | 113.70$^{ab}$ ±3.19 | 78.57$^{bcd}$ ±1.57 |

[1] Percentage of control: DMD and CMCase and xylanase activities of the control treatment were taken as 100%.
Each value represents the mean±SE of triplicate cultures.
$^{a,b,c,d,e}$ Means in the same column with different superscripts differ significantly ($p<0.05$).

with increasing chain length (Smith and Lough, 1976; Lough and Smith, 1976). We suggest that the relationships between the melting point of fatty acids and cellulolysis by fungus indicates that hard fats (i.e., those with high melting points) are more inert *in vivo* and *in vitro*, and thus, less likely to coat either microbial cells or feed particles.

At higher concentrations of fatty acids the inhibition may be of a physicochemical nature, with the surface-active fatty acids adhering to the fungal cell wall and impeding the passage of essential nutrients. In support of this, the greatest inhibition was observed at fatty-acid concentrations where there was an insoluble fatty acid phase, under which conditions the smearing of fungal cells by fatty acids would seem more likely.

Unexpectedly the addition of fatty acids at lower concentrations (0.01 and 0.001%) did not inhibit FP cellulose degradation or CMCase or xylanase activities. FP cellulose degradation and CMCase activity by the anaerobic fungus were slightly increased by the addition of fatty acids (without statistical significance) (table 6). The results obtained in this study are, in general, inconsistent with the major effects observed when fatty acids and/or oils are added to pure cultures of anaerobic bacteria and protozoa (Henderson, 1973; Maczulak et al., 1981).

The reasons for enhanced cellulolysis by fatty acid at lower concentrations, but not higher concentrations in fungal cultures, are not clear. These effects may be related to lactate production, which in high concentrations is known to exert a negative effect on cellulolysis. Another possible explanation is that fatty acid uptake by fungi may provide fatty acids as growth factors for fungus. Orpin and Letcher (1979) showed that certain unsaturated fatty acids can stimulated the *in vitro* growth of *N. frontalis*. In practical feeding conditions, the concentration of unsaturated fatty acids is very low in poor hay diets (Dawson and Kemp, 1970) and uptake by fungi may provide a possible means whereby fatty acids may escape hydrogenation in the rumen (Dawson and Kemp, 1970; Kemp et al., 1975). This would increase the amount available to satisfy the host's small requirement for these essential fatty acids (Lindsay and Leat, 1977).

Medium- and long-chain fatty acids have long been recognized as inhibitors of spores, vegetative bacterial cells, protozoa and some aerobic fungi as well as of cellulolysis *in vivo* and *in vitro*. However, the findings of our study based on FP cellulose degradation, enzymatic activities and

VFA production confirm that cellulolysis by anaerobic fungi is not inhibited by the addition of lower levels (less than 0.01% in the medium) of fatty acids. This is in disagreement with numerous reports which concluded that other rumen microorganisms (bacteria and protozoa) are greatly inhibited by oils and fatty acids (Henderson, 1973; Maczulak et al., 1981). The present study has also shown, for the first time, that cellulolysis by ruminal fungi is markedly inhibited when fungus are grown in medium supplemented with higher levels of fatty acids. The cellulolysis by the anaerobic gut fungal strains was stimulated by low levels of fatty acids is intriguing, as this observation contradicts the theory which suggests that the growth of cellulolytic bacteria (*B. flavefaciens, R. albus* and *R. flavefaciens*) and protozoa strongly inhibited by lower levels of fatty acids.

In conclusion, this is the first report examining the effects of fatty acids on the anaerobic gut fungi; the lower levels of fatty acids used in this experiment were able to stimulate the growth of fungi, but higher levels of fatty acids greatly inhibited fungal cellulolysis. We are currently conducting research in an attempt to understand the mechanism of fatty acid stimulation and/or inhibition of ruminal fungi.

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