Effect of Medium Chain Fatty Acids and their Derivatives on *in vitro* Rumen Fermentation

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The use of fat for milking cows has been considered a useful supplement to reduce dietary cereal content¹⁵⁾. However, excess fat disturb the rumen function¹⁴⁾. Therefore, the use of so-called rumen-protected "by-pass" fat has been developed in the past two decades, and their effectiveness in high-producing cow has been demonstrated^{6,11)}. These compounds are made of long chain fatty acids (LCFAs), but medium chain fatty acids (MCFAs; C₆-C₁₂) are also available as by-products from the chemical industry and can possibly be used as feed additives for ruminants. However, the use of MCFAs or medium chain triglycerides (MCTs) has been limited to medical uses or infant (preruminant) feeding²⁻⁴⁾. Therefore information on their effect, especially of their rumen-protected form on rumen fermentation is still very limited. This experiment was conducted to compare the responses of the *in vitro* rumen microbial system to MCFAs as free acids, as triglycerides and as calcium salts (rumen-protected form) using a batch fermentation system with four different carbohydrate

substrates.

Materials and Methods

An *in vitro* batch fermentation system was used to test the effects of MCFAs and their derivatives¹⁸⁾. Substrates were reagent grade cellulose powder (Nacalaitesque, Kyoto), maize starch (Nacalaitesque, Kyoto), oat spelt xylan (Aldrich, Milwaukee, USA) and citrus pectin (Wako Pure Chemical, Osaka). Three different forms (free fatty acid (FA), calcium-salt (CA) and triglyceride (TG)) of two MCFAs (*n*-caprylic (C₈) and *n*-capric (C₁₀) acids) were examined. All forms of MCFAs were obtained from Kao Co. (Tokyo). The *in vitro* fermentations were conducted with 1 g substrate, 40 ml of strained rumen fluid, 160 ml of carbon dioxide-saturated McDougall buffer and 2 ml of ammonium sulfate (17.6% (w/v)). Each form of the MCFA derivatives was added at 10% (w/w) of substrate as FA except for the control flask. MCFA-derivatives are solid at room temperature except for C₈-FA and -TG. Solid compounds were scraped and added to

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the flasks, liquid compounds were added as droplets. The initial gas phase was 100% carbon dioxide.

A rumen-cannulated adult wether was used as the rumen fluid donor. This sheep received 400 g timothy hay and 200 g of a commercial concentrate mainly containing maize (47%), wheat bran (39%), soybean cake (8%) (Coop dairy-14, Kumiai Shiryo, Kobe) at 09:00 and 18:00 h. Water and mineral blocks were available to the sheep at all times. The rumen fluid was collected via a cannula just before morning feeding and strained through four layers of surgical gauze. Since the fermentation system was composed of eight units (flasks), four treatments (no-MCFA-Control, FA, CA and TG) of each MCFA (C_8 or C_{10}) were tested duplicate. In addition to those, another two control flasks were prepared for 0-h determinations. MCFAs (C_8 or C_{10}) were tested on one of four substrates and each batch was tested on two different days. Consequently, 16 batches of incubation (two MCFAs $\times$ four substrates $\times$ 2) were made. Starch was incubated for 24 h and the other three substrates were incubated for 48 h with continual shaking at 39°C. Fermentation was halted by cooling in a crushed ice bath followed by centrifugation at $1,500 \times g$ for 30 min. The flasks for 0-h determination were treated in the same manner immediately after introduction of the inoculum.

Volatile fatty acids (VFA) in supernatant and the collected gas during incubation were quantitatively analyzed by GLC¹⁶⁾. Production of VFA was calculated as the difference between quantities at 48-h or 24-h vs. 0-h. Pellets from cellulose and xylan fermentations were treated by neutral-detergent (ND) to estimate residual substrates (cellulose and ND-insoluble xylan (NDIX))¹⁷⁾. Another series of incubations were carried out in order to determine the effect of MCFAs on starch, pectin and total xylan digestibilities. Each batch was also repeated on two different days.

After fermentation was stopped by cooling in a crushed ice bath, residual xylan, starch and pectin were precipitated by ethanol and centrifuged at $1,500 \times g$ for 30 min. The initial ethanol concentrations (% (v/v)) were 80 for the former two substrates and 88 for pectin. The pellets were washed twice with 80 or 88% ethanol and successively washed with 90%, 99.5% and 100% ethanol and finally with diethylether. Pellets were dried in a desiccator under vacuum. Ethanol-precipitated xylan (EPX) was then determined by the method of KOEHLER¹³⁾, and starch was extracted by perchloric acid and then measured as free glucose¹⁾. Pectin was measured as uronic acid after acid hydrolysis¹⁴⁾. The digestibilities of the substrates were calculated using 0-h values as the initial amounts.

The values of digestibilities, gas and VFA within the same MCFA-treatment were analyzed by analysis of variance (Sources of variation were treatment, experimental day and interaction) and, where appropriate, the values were analyzed by Tukey's test to identify treatment difference⁹⁾.

Results and Discussion

Table 1 shows the effects of MCFAs and their derivatives on carbohydrate digestion *in vitro*. Cellulose digestion was inhibited by all the MCFA-treatments. Although the effect was statistically significant ($p < 0.05$), the effect of C_8 was relatively small. There was virtually no difference between C_8 -treatments. While the effects of C_{10} were more apparent than those of C_8 . None of C_8 -treatments significantly affected xylan digestion. While C_{10} -treatments significantly reduced xylan digestion and the digestion of NDIX was more affected than that of EPX. The subpopulation which was involved in NDIX-digestion was likely to include different organisms from those in EPX-digestion (or more precisely ND-insoluble xylan digestion) and the former subpopulation was possibly more affected by C_{10} -

Table 1. Effect of medium chain fatty acids on carbohydrate digestion (% digested during incubation¹⁾) *in vitro*²⁾

	Caprylic acid					Capric acid				
	Control ³⁾	CA	TG	FA	SEM	Control	CA	TG	FA	SEM
Cellulose	92.3 ^a	86.9 ^b	89.2 ^b	90.7 ^{ab}	0.41	92.1 ^a	41.6 ^b	66.7 ^c	25.9 ^d	2.43
Xylan										
NDIX ⁴⁾	82.0	79.6	81.5	81.3	0.47	80.4 ^a	49.6 ^b	56.3 ^b	24.7 ^c	1.34
EPX ⁵⁾	92.1	91.0	92.8	92.3	0.33	92.0 ^a	71.6 ^b	94.0 ^a	75.0 ^c	0.52
Pectin	72.8	68.5	73.4	70.9	1.55	71.5	70.4	75.6	70.5	1.18
Starch	98.6	98.8	98.8	99.4	0.10	98.8	98.9	98.8	98.7	0.05

¹⁾ Starch was incubated for 24 h, other three substrates were for 48 h.

²⁾ Values within a same row within a same batch of MCFA sharing different superscripts differ significantly ($p < 0.05$).

³⁾ Control, no MCFA; CA, Ca-soap; TG, triglyceride; FA, free acid.

⁴⁾ Neutral detergent insoluble xylan.

⁵⁾ Ethanol-precipitated xylan (=total xylan).

treatments. Partial solubilization of hemicellulose by ND-treatment is well known. Indeed, ND-treatment solubilized a significant part (approximately 40%) of xylan used in this experiment. This suggests that physico-chemical properties of NDIX may be different from those of ND-soluble xylan and such difference in structure could select a specific population for each xylan fraction. Unlike cellulose and xylan digestion, none of the MCFAs-treatments significantly affected the starch and pectin digestion indicating that amylolytic and pectinolytic subpopulations are less sensitive to MCFAs than cellulolytic and hemicellulolytic subpopulations. The fermentation pattern (Data not shown) was not significantly affected except for a few cases. Carbon dioxide production was affected by C₁₀-treatments, particularly by FA, in cellulose and xylan fermentation (20 to 80% reduction from control). Methane production from cellulose was significantly depressed by C₁₀-FA (60% reduction from control), but it was unaffected or slightly increased in other cases. Acetate production was significantly depressed by C₁₀-treatments in cellulose and xylan fermentation (20 to 50% reduction from control). Propionate production from cellulose was depressed by all the treatments, and the effect of C₁₀ was

the most apparent. Butyrate production was affected by the treatments in a similar manner to methane production. The published information is very limited on the effect of MCFAs on rumen fermentation; the earlier report of BLAXTER and CZERKAWSKI⁷⁾ and recent report of GRUMMER and SOCHA¹⁰⁾ are only ones available. The former two authors reported that C₁₀-FA is three times more inhibitory than C₈-FA to ruminal methanogenesis and digestion of dietary non-lipid components. Present results principally agree with their earlier observations⁷⁾ especially those concerned with fiber digestion, but no inhibitory effect of MCFAs on methanogenesis was detected except for cellulose fermentation in this experiment. This suggests that MCFAs are not necessarily toxic to methanogen and antimethanogenic effect of MCFAs may depend on dietary conditions. BLAXTER and CZERKAWSKI have concluded that rumen methanogens are sensitive to FA, but this hypothesis has not been further confirmed in the methanogenic monoculture system and in *in vivo* trials under various feeding conditions. GRUMMER and SOCHA have not observed any change in acetate and propionate concentration in the rumen of cows maintained on a mixed diet supplemented with MCT, but have observed a slight increase in

butyrate concentration in the rumen. However, the response of rumen microorganisms to MCT was not measured and no explanation for the fermentation-shift was given. The reasons for augmentation of butyrate are still unclear. Unlike butyrate and methane, reduction in acetate and propionate production in cellulose and xylan fermentation may well be explained by the reduced substrate utilization.

Fatty acids, MCFAs and unsaturated LCFAs in particular, are known to have antimicrobial activities¹²⁾, but the mode of action against rumen microorganisms has not been established conclusively⁸⁾. The adsorption of fatty acid or fat onto feed particles and microorganisms is usually considered as the major cause of inhibition of ruminal fiber digestion. CHALUPA *et al.*⁸⁾ stressed that the melting point of LCFAs determines its toxicity against the rumen microbial system. They demonstrated that LCFAs of lower melting point are more inhibitory to rumen fermentation. Melting points of the compounds which were tested in this experiment, except for C_8 - and C_{10} -CA, are lower than the temperature at which incubation was run. Therefore TG and FA of both acids were likely to disperse in the culture medium during incubation, and thus, to adsorb on feed particles and microorganisms. Unlike LCFAs, toxicity of C_{10} was higher, although its melting point is higher than C_8 . The reasons for higher toxicity of C_{10} are still unknown, but it is unlikely that the melting point was the first determining factor in the case of MCFAs. The structure of C_{10} (e.g. the chain length which affects the affinity to the membrane) may cause a higher antimicrobial activity.

The effect of derivatization of LCFAs on rumen fermentation was studied by CHALUPA *et al.*⁸⁾ who observed that both CA and TG are virtually inert to rumen fermentation. Such derivatizations are totally effective in the prevention of toxicity even with unsaturated LCFAs. They suggested that insolubility of

CA in rumen liquid, a lower binding capacity of CA and TG to feed particles and bacteria, and a limited microbial activity which releases little FA from CA and TG are the major causes for the inertness. However, both types of derivatization failed to prevent the inhibitory action of C_{10} to fiber digestion in this experiment. Such incomplete prevention of inhibitory effect might be explained, if binding capacity of C_{10} -TG to feed particles and bacteria was high or if the present microbial system was highly lipolytic (or MCT are more readily hydrolyzed than LCFA). The reasons for incomplete prevention of inhibitory effect by CA are also complex. C_{10} -CA was virtually insoluble to culture fluid; indeed, a significant amount of C_{10} -CA appeared to remain intact after 48 h incubation. CA, in general, releases FA at low pH, but the pH of culture fluid after incubation ranged between 5.8 and 6.2. Such pH is not low enough to produce FA from CA. There are two possibilities which explain the results; the present microbial system could hydrolyse C_{10} -CA to release sufficient amount of FA or C_{10} -CA itself was toxic. Although the causes of toxicity are unclear, the present results suggest that the effect of C_{10} -CA on rumen fermentation should carefully be examined before the practical usage of MCFAs-derivatives. Indeed, our recent *in vivo* experiment¹⁹⁾ indicated that CA of mixed MCFA ($C_8 : C_{10} = \text{ca. } 1 : 1$) significantly reduced fungal and protozoal population sizes, and consequently fiber digestion in rumen, when the compound was given to sheep at 5% of daily diet.

The present results suggest that C_8 and its derivatives would not cause detrimental effects on rumen fermentation (especially fiber fermentation). While C_{10} had clear negative effects on fiber digestion even with its rumen protected form. Reduction of C_{10} content in the MCFA-mixture may be recommended in the practical use of MCFAs, but information is still limited (e.g. an optimum dose, a physical form of products etc.). Further studies are

required in field experiments in order to examine their effects on rumen microbial population and feed utilization of ruminants under production conditions.

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