

Effect of caprylic, capric and oleic acid on growth of rumen and rabbit caecal bacteria*

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ABSTRACT

In a search for alternatives of in-feed antibiotics, the antimicrobial activity of caprylic (C8:0), capric (C10:0) and oleic (C18:1) acid was investigated in pure cultures of 19 strains of rumen and rabbit caecal bacteria, and in incubations of the rumen and rabbit caecal contents. In glucose-grown bacterial cultures the minimum inhibitory concentration (MIC) of caprylic acid ranged from 1 to 3 $\mu\text{l}\cdot\text{ml}^{-1}$. Two strains of *Bacteroides ovatus* were less susceptible to capric than to caprylic acid. In other strains, the MIC of capric acid was 0.25–0.50 $\mu\text{l}\cdot\text{ml}^{-1}$. The growth of most strains was not much affected by oleic acid. An exception to this were rumen bacteria *Butyrivibrio fibrisolvens* (MIC from < 0.05 to 1 $\mu\text{l}\cdot\text{ml}^{-1}$) and *Lachnospira multiparus* (MIC of 0.25 to 1 $\mu\text{l}\cdot\text{ml}^{-1}$). In incubations of the rumen and caecal contents caprylic and capric acid decreased the production of volatile fatty acids and gas, and increased production of lactate. In latter incubations the inhibitory effects of caprylic and capric acid were similar. In incubations of the rumen contents, capric acid was more efficient than caprylic acid when supplied at low concentrations (<1.25 $\mu\text{l}\cdot\text{ml}^{-1}$), but less efficient when supplied at 2.5 and 5 $\mu\text{l}\cdot\text{ml}^{-1}$. Effects of oleic acid in rumen and caecal cultures were not significant, except the increase in production of lactate by rumen microorganisms. It can be concluded that microorganisms of the animal digestive tract are susceptible to inhibition by caprylic and capric acid added to microbial cultures at fairly low concentrations. Oleic acid was far less effective.

KEY WORDS: fatty acids, rumen, rabbit caecum, bacteria, inhibition, fermentation

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INTRODUCTION

Antimicrobial activity of fatty acids is well known. In the presence of fatty acids, the transport of protons through microbial membranes becomes uncontrolled. Consequently, fatty acids interfere with the metabolism of energy within the cell and disturb energy-dependent processes, e.g., active transport of nutrients into bacterial cells, maintenance of concentration gradients, etc. (Galbraith and Miller, 1973). Results of various investigations of the antimicrobial activity of fatty acids showed that (i) Gram-positive bacteria were more susceptible to the action of fatty acids in minute concentrations than Gram-negative bacteria, (ii) inhibitory properties of unsaturated fatty acids were more pronounced than those of saturated acids, and (iii) antibacterial activity of saturated fatty acids was optimal for a chain length around C12 (reviewed by Nieman, 1954). Several authors examined effects of fatty acids on rumen bacteria. Henderson (1973) found that fatty acids containing 10 to 18 carbon atoms inhibited ruminococci, butyrivibrios and production of methane in cultures of *Methanobacterium ruminantium*. No effect on growth of Gram-negative bacteria *Anaerovibrio lipolytica*, *Peptostreptococcus* (now *Megasphaera*) *elsdenii*, *Selenomonas ruminantium* and *Bacteroides* (now *Prevotella*) *ruminicola* was observed. Oleic acid was the most inhibitory of the series of acids. In experiments of Maczulak et al. (1981) the growth of *Ruminococcus albus* and *Ruminococcus flavefaciens* was almost completely inhibited at oleic acid concentration as low as 5 mg·l⁻¹. This concentration of oleic acid inhibited also the growth of one out of five strains of *Butyrivibrio fibrisolvens*. The growth of other butyrivibrios was not much affected by oleic acid. Low concentrations of oleic acid enhanced growth of *S. ruminantium* and *B. ruminicola*. In most strains, palmitic and stearic acid had little effect on growth. In mixed cultures of rumen microorganisms lauric acid (C12:0) decreased production of volatile fatty acids to a greater extent than myristic, palmitic, stearic, oleic, linoleic and arachidic acid (Chalupa et al., 1984). Lauric acid increased, but other acids decreased the acetate to propionate molar ratio.

Few studies deal with bacteriocidal effects of medium-chain fatty acids. Smith (1965) reported that suckling rabbits were unique among young of seven animal species tested in that the contents of their stomach and small intestine were almost completely sterile. To explain this finding, Canas-Rodriguez and Smith (1966) suggested that the rabbit milk fat contained antimicrobial compounds, identified as eight- and ten-carbon saturated fatty acids (caprylic and capric acid, respectively). These acids are the principal fatty acids in the rabbit milk. Typically, caprylic and capric acid represent from one third to one half of the total fatty acids in the rabbit milk fat (Christ et al., 1996; Lebas et al., 1996). Both acids are practically absent from the feed, thus, rabbits synthesize them in the mammary gland. The antimicrobial activity of rabbit milk was confirmed by Marounek et al. (1999). Rabbit

milk added at 4.8% (v/v) significantly decreased production of microbial metabolites in cultures of rabbit caecal contents, whereas no inhibitory effect of a corresponding mixture of cow milk fat, casein and lactose was observed. Capric acid at a concentration higher than 1.2 to 1.6 g·l⁻¹ virtually completely killed the acetogenic and methanogenic population of methanogenic sludge (Rinzema et al., 1994). Matsumoto et al. (1991) found that capric acid was the most toxic for rumen protozoa among six fatty acids tested. Progressively less inhibition was displayed with either an increase or a decrease in the carbon chain length. An adverse effect of caprylic and capric acid on rumen protozoa was observed also by Dohme et al. (2001). Some authors have reported the inactivation of lipid-enveloped viruses by caprylic and capric acid (Lundblad and Seng, 1991; Isaacs et al., 1995).

Antimicrobial substances are often used to stimulate growth of farm animals and for the control of enteritis infections. However, most of antimicrobial performance promoters were banned recently, and the use of antibiotics for enteritis prevention has been viewed critically. Thus, there is a need for alternatives to antibiotics, usable in animal production and acceptable by consumers. The purpose of this study was to evaluate the antibacterial activity of caprylic, capric and oleic acid using pure strains of rumen and rabbit caecal bacteria, and in incubations of rumen and rabbit caecal contents.

MATERIAL AND METHODS

Bacteria, media and experimental procedure

Rumen bacteria *Eubacterium limosum* ATCC 8486 and *Lachnospira multiparus* ATCC 19207 were supplied from the American Type Culture Collection. Other rumen bacteria (*Butyrivibrio fibrisolvens* 670, AR11 and OR38b, *Lactobacillus acidophilus* 30 and 51, *Megasphaera elsdenii* LC1, *Prevotella ruminicola* AR20 and AR29, *Ruminococcus albus* SY3, *Selenomonas ruminantium* 625, *Streptococcus bovis* X4 and *Veillonella alcalescens* 692¹) were available in the culture collection of the Institute of Animal Physiology and Genetics. *L. acidophilus* strains were isolated from the rumen of young calves, other bacteria from the rumen of adult ruminants. *Bacteroides caccae* KWN, *Bacteroides ovatus* PB2 and PB7, and *Bifidobacterium pseudolongum* P2 and P6 were isolated from the rabbit caecum. Rumen bacteria were grown on a medium containing (per litre): K₂HPO₄ · 3H₂O - 5.9 g, KH₂PO₄ - 4.5 g, NaHCO₃ - 3.0 g, (NH₄)₂SO₄ - 2.9 g, NaCl - 0.9 g, MgSO₄ · 7 H₂O - 0.09 g, CaCl₂ - 0.09 g, yeast extract - 3.0 g, bactopectone - 3.0 g, glucose - 6.0 g, clarified rumen fluid - 100 ml. A trace metal solution (1 ml) containing nitrilotriacetic acid (Clark and Holms, 1976) was also added. *V. alcalescens* was grown on sodium pyruvate (18 g·l⁻¹) instead of glucose. Rabbit intestinal bacteria were

grown on the same medium, except that the rumen fluid was replaced by caecal extract prepared by autoclaving (110°C, 45 min) equal quantities of the rabbit caecal contents and distilled water. The extract was clarified by centrifugation.

Media were dispensed to gas-tight glass flasks, and caprylic, capric and oleic acid (Sigma) added at 0, 0.05, 0.1, 0.25, 0.5, 1, 2, 3 and 5 $\mu\text{l}\cdot\text{ml}^{-1}$, together with an equivalent amount of 5 M NaOH. Capric acid was heated to 40°C before pipetting. Media were supplemented with 0.05% cysteine·HCl. Flasks were filled with CO₂, closed with rubber stoppers and autoclaved at 110°C for 45 min. Inoculated cultures were incubated in triplicate at 39°C for 24 h. Then the cultures were examined for visible growth and pH was measured. The minimum inhibitory concentration (MIC) was the lowest concentration of caprylic and capric acid that prevented the visible growth and pH drop in treated cultures (typically from 6.6 to 5.8 in controls). Media with oleic acid were turbid, thus residual glucose was determined using a commercial kit from Lachema (Brno, Czech Republic). The MIC was the lowest concentration of oleic acid that prevented glucose utilization in treated cultures.

Cultivation of rumen and caecal contents

Rumen contents were obtained from two dry fistulated cows fed 1 kg of a commercial concentrate per day, 2 kg lucerne hay and maize silage *ad libitum*. The rumen content was homogenized in a laboratory blender under CO₂ atmosphere for 2 min. Ten ml of the homogenate were added to 30 ml of warm (39°C) Burroughs buffer (Burroughs et al., 1950) in 100 ml incubation flasks (serum bottles) containing wheat bran (0.8 g), yeast extract (40 mg), urea (20 mg) and sodium sulphide (20 mg) as a reducing agent. The NaHCO₃ concentration in the buffer was increased to 0.07 M, to prevent a pH drop below 6.0 and 5.5 in rumen and caecal cultures, respectively. In the first experiment, caprylic and capric acid were added at 0, 0.625, 1.25, 2.5 and 5 $\mu\text{l}\cdot\text{ml}^{-1}$, together with an equivalent amount of NaOH. In the second experiment, oleic acid was added at 0, 2 and 5 $\mu\text{l}\cdot\text{ml}^{-1}$. The flasks were flushed with CO₂, hermetically closed with rubber stoppers and incubated at 39°C for 8h in a shaking water bath. Each control or experimental arrangement was incubated in four replicates.

The same experimental procedure was used for incubation of the rabbit caecal content. Three-month-old rabbits (Hyla 2000 genotype) were fed *ad libitum* a granulated concentrate containing dehydrated lucerne, wheat bran, extracted sunflower meal, barley, oat and a vitamins-minerals supplement. Four rabbits were slaughtered, their caeca emptied and pooled caecal contents were used for inoculation of cultures as described above.

At the end of incubations, manometric pressure in the incubation vessels was measured. Samples of the incubation fluid from the beginning and the end of incubation were analysed. Total volatile fatty acids (VFA) were estimated by titra-

tion, after steam distillation. Lactic acid was assayed by the microdiffusion method (Conway, 1957).

RESULTS

Minimum inhibitory concentrations of caprylic and capric acid in 19 bacterial strains tested are shown in Table 1. In 17 strains, the MIC of caprylic acid was higher than that of capric acid. Concentrations around $2.0 \mu\text{l}\cdot\text{ml}^{-1}$ inhibited bacterial growth in caprylic acid-treated cultures. *B. ovatus* was the only bacterium less

TABLE 1
Minimum inhibitory concentrations of caprylic, capric and oleic acid in cultures of fourteen rumen and five rabbit caecal bacteria*

Bacterium	Gram staining	MIC, $\mu\text{l}\cdot\text{ml}^{-1}$		
		caprylic	capric	oleic
<i>Butyrivibrio fibrisolvens</i> 670	+	1	0.25	0.25
<i>Butyrivibrio fibrisolvens</i> AR11	+	1	0.5	< 0.05
<i>Butyrivibrio fibrisolvens</i> OR38b	+	2	0.5	1
<i>Eubacterium limosum</i> ATCC 8486	+	3	0.5	> 5
<i>Lachnospira multiparus</i> ATCC 19207	+	1	0.5	0.25
<i>Lactobacillus acidophilus</i> 30	+	2	0.5	> 5
<i>Lactobacillus acidophilus</i> 51	+	3	0.5	> 5
<i>Megasphaera elsdenii</i> LC1	-	2	0.25	> 5
<i>Prevotella ruminicola</i> AR20	-	2	0.5	> 5
<i>Prevotella ruminicola</i> AR29	-	1	0.25	5
<i>Ruminococcus albus</i> SY3	+	2	0.5	> 5
<i>Selenomonas ruminantium</i> 625	-	2	0.25	> 5
<i>Streptococcus bovis</i> X4	+	3	0.25	> 5
<i>Veillonella alcalescens</i> 692 ¹	-	2	0.25	nd
<i>Bacteroides caccae</i> KWN	-	2	0.5	> 5
<i>Bacteroides ovatus</i> PB2	-	2	5	> 5
<i>Bacteroides ovatus</i> PB7	-	3	5	> 5
<i>Bifidobacterium pseudolongum</i> P2	+	2	0.5	> 5
<i>Bifidobacterium pseudolongum</i> P6	+	2	0.25	> 5

* control and treated cultures were incubated in triplicate
nd, not determined

susceptible to capric than to caprylic acid. In other strains the MIC of capric acid ranged from 0.25 to $0.5 \mu\text{l}\cdot\text{ml}^{-1}$. Gram-positive and Gram-negative bacteria did not differ in sensitivity to caprylic acid. Similarly, there was no significant difference

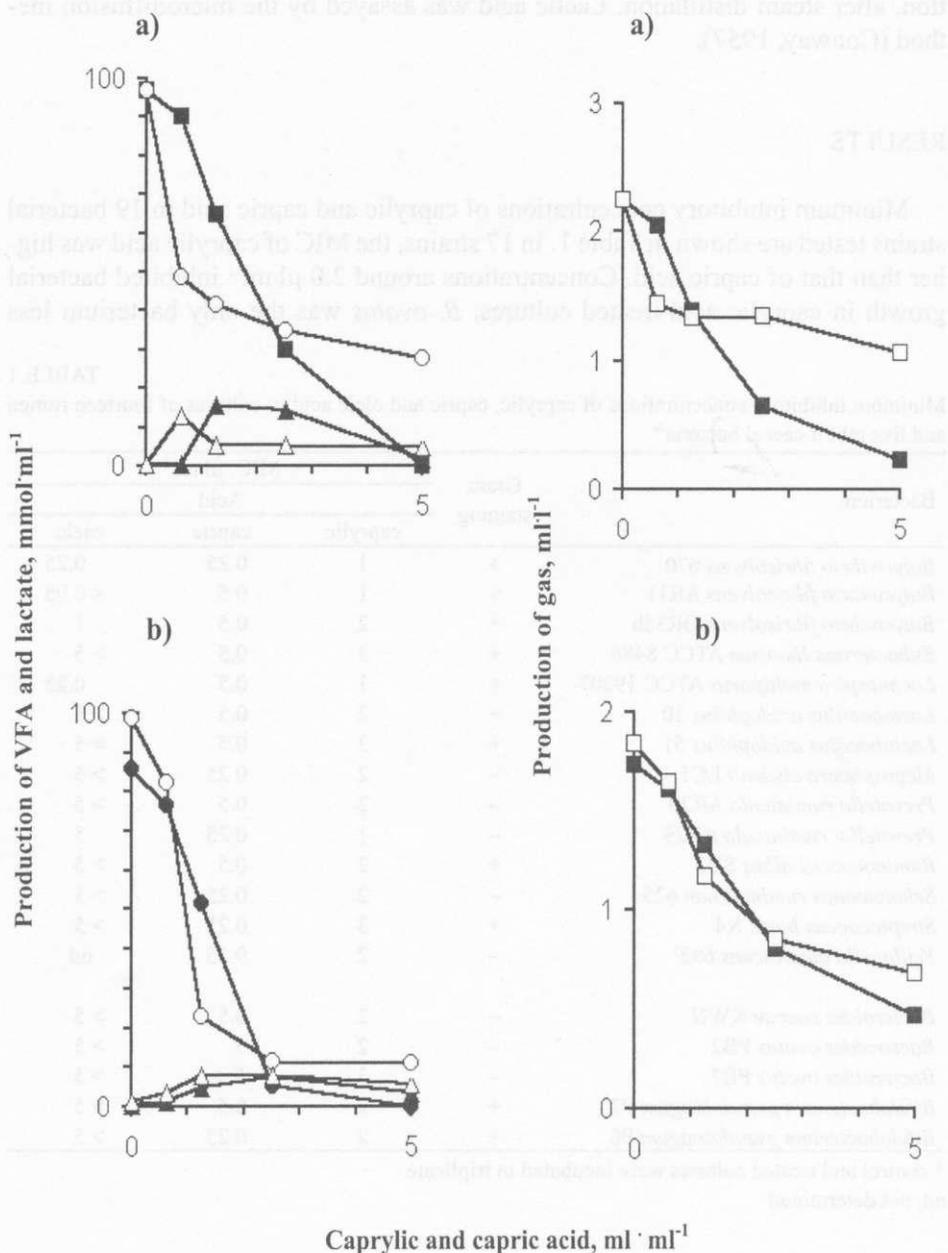


Figure 1. Effect of caprylic and capric acid on production of VFA (\bullet , \circ), lactate (\blacktriangle , \triangle) and gas (\blacksquare , \square) in cultures of rumen (a) and rabbit caecal contents (b). Closed symbols, caprylic acid; open symbols, capric acid. All cultures were incubated in four replicates

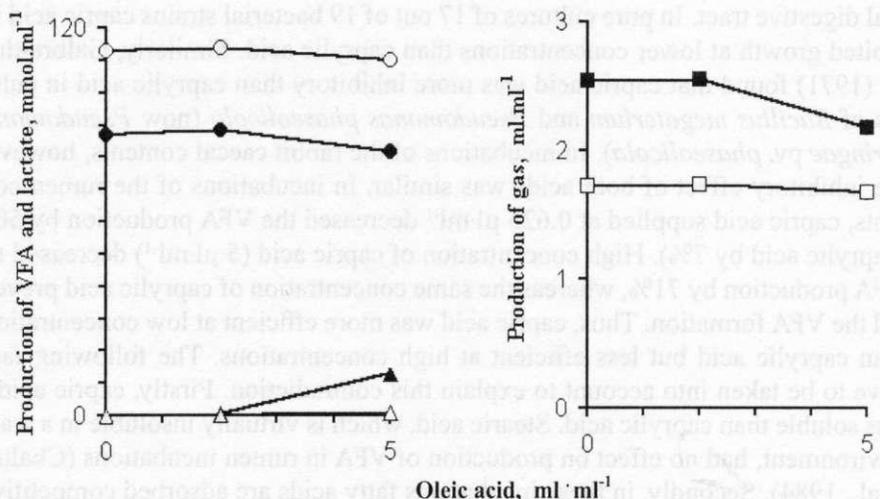


Figure 2. Effect of oleic acid on production of VFA (●, ○), lactate (▲, △) and gas (■, □) in cultures of rumen and rabbit caecal contents. Closed symbols, rumen cultures; open symbols, caecal cultures. All cultures were incubated in four replicates

in sensitivity of Gram-positive and Gram-negative bacteria other than *B. ovatus* to caprylic acid. Most of bacterial strains tested were resistant to oleic acid. Fairly low concentrations of oleic acid, however, inhibited growth of butyrvibrios (MIC from < 0.05 to 1 $\mu\text{l}\cdot\text{ml}^{-1}$) and *L. multiparus* ATCC 19207 (MIC of 0.25 $\mu\text{l}\cdot\text{ml}^{-1}$).

Effects of caprylic and capric acid in mixed cultures of rumen and caecal microorganisms are shown in Figure 1. Both caprylic and capric acid decreased the VFA and gas production and increased production of lactate. In rumen cultures, caprylic acid was less efficient than capric acid when supplied at low concentrations (0.625 and 1.25 $\mu\text{l}\cdot\text{ml}^{-1}$). The high caprylic acid concentration (5 $\mu\text{l}\cdot\text{ml}^{-1}$), however, completely inhibited production of microbial metabolites, whereas capric acid at 5 $\mu\text{l}\cdot\text{ml}^{-1}$ apparently allowed a moderate microbial growth. In rabbit caecal cultures effects of both acids were similar. Oleic acid had a negligible effect on VFA and gas production, but increased production of lactate in rumen incubations from 0 to 12.9 $\mu\text{mol}\cdot\text{ml}^{-1}$ (Figure 2).

DISCUSSION

Antibacterial effects of caprylic and capric acid have generally received less attention than those of long-chain fatty acids. In the present study, caprylic and capric acid were shown to inhibit growth of various bacteria isolated from the ani-

mal digestive tract. In pure cultures of 17 out of 19 bacterial strains capric acid inhibited growth at lower concentrations than caprylic acid. Similarly, Galbraith et al. (1971) found that capric acid was more inhibitory than caprylic acid in cultures of *Bacillus megaterium* and *Pseudomonas phaseolicola* (now *Pseudomonas syringae* pv. *phaseolicola*). In incubations of the rabbit caecal contents, however, the inhibitory effect of both acids was similar. In incubations of the rumen contents, capric acid supplied at $0.625 \mu\text{l}\cdot\text{ml}^{-1}$ decreased the VFA production by 50% (caprylic acid by 7%). High concentration of capric acid ($5 \mu\text{l}\cdot\text{ml}^{-1}$) decreased the VFA production by 71%, whereas the same concentration of caprylic acid prevented the VFA formation. Thus, capric acid was more efficient at low concentrations than caprylic acid but less efficient at high concentrations. The following facts have to be taken into account to explain this contradiction. Firstly, capric acid is less soluble than caprylic acid. Stearic acid, which is virtually insoluble in a water environment, had no effect on production of VFA in rumen incubations (Chalupa et al., 1984). Secondly, in these incubations fatty acids are adsorbed competitively onto feed particles and bacteria (Harfoot et al., 1974). The adsorption of fatty acids decreases with increasing unsaturation (Harfoot et al., 1974), and increases with the chain length (Neys and Joos, 1998). Preferential binding to feed particles decreases toxicity of fatty acids towards rumen and caecal bacteria. Inclusion of powdered cellulose in the medium reversed the inhibitory effect of added fatty acids (Maczulak et al., 1981). Oleic acid, which was inhibitory in pure cultures of some rumen bacteria, influenced production of microbial metabolites in our rumen and caecal incubations only marginally.

As far as we know, no previous study examined effect of fatty acids on lactate production. In the present experiments, in rumen and caecal cultures fatty acids tested increased production of lactate. This may be caused either by proliferation of lactate producers or by inhibition of lactolytic microflora.

It follows from our results that caprylic and capric acid are efficient antimicrobials both in pure bacterial cultures grown on glucose and in digesta samples incubated *in vitro*. Their growth-promoting effect in the animal, however, is not certain as both acids inhibited growth of Gram-positive and Gram-negative bacteria at similar concentrations. Most of antibacterial growth promoters are substances active against Gram-positive bacteria (Brander, 1982). Further experiments *in vivo*, thus, should decide if these acids are suitable as feed additives for young animals in the post-weaning period, e. g., for early weaned rabbits. Experiments *in vivo* should also assess activity of caprylic and capric acid against parasitic protozoa of the digestive tract.

CONCLUSIONS

The results presented here confirm significant antimicrobial activity of caprylic and capric acid against microorganisms of the animal digestive tract. Antimicrobial activity of oleic acid was much lower. The antimicrobial efficiency of caprylic and capric acid differed in pure bacterial cultures grown on glucose and in incubations of digesta samples, probably because of the presence of solid particles in latter cultures. Experiments are needed to assess *in vivo* effects of both acids.

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STRESZCZENIE

Wpływ kwasów kaprylowego, kaprynowego i oleinowego na rozwój bakterii żwacza oraz jelita ślepego królika

W poszukiwaniu alternatywy dla antybiotyków paszowych, badano anty-drobnoustrojową aktywność kwasów: kaprylowego (C8:0), kaprynowego (C10:0) oraz oleinowego (C18:1) w czystych kulturach 19 szczepów bakterii żwacza i jelita ślepego królika. W kulturach bakterii hodowanych na pożywce glukozowej, minimalne stężenie hamujące (MIC) kwasu kaprylowego wahało się od 1 do 3 $\mu\text{l}\cdot\text{ml}^{-1}$. Dwa szczepy *Bacteroides ovatus* były mniej podatne na działanie kwasu kaprynowego niż kaprylowego. U innych szczepów MIC kwasu kaprynowego wynosiło 0,25-0,5 $\mu\text{l}\cdot\text{ml}^{-1}$. Kwas oleinowy miał mały wpływ na rozwój większości szczepów. Wyjątkiem były tylko bakterie żwaczowe *Butyrivibrio fibrisolvens* (MIC od <0,05 do 1 $\mu\text{l}\cdot\text{ml}^{-1}$) oraz *Lachnospira multiparus* (MIC od 0,25 do 1 $\mu\text{l}\cdot\text{ml}^{-1}$). Podczas inkubacji treści żwacza i jelita ślepego kwasy kaprylowy i kaprynowy obniżały produkcję lotnych kwasów tłuszczowych i gazu, a zwiększały produkcję kwasu mlekowego. Wpływ hamujący tych dwóch kwasów był podobny. Podczas inkubacji treści żwacza działanie kwasu kaprynowego było efektywniejsze niż kaprylowego gdy dodawano je w mniejszym stężeniu (<1,25 $\mu\text{l}\cdot\text{ml}^{-1}$), odwrotnie – przy większym stężeniu (2,5 do 5 $\mu\text{l}\cdot\text{ml}^{-1}$) było mniej efektywne. Działanie kwasu oleinowego na kultury żwacza i jelita ślepego nie było istotne, z wyjątkiem zwiększenia produkcji kwasu mlekowego przez drobnoustroje żwacza.

W podsumowaniu można stwierdzić, że drobnoustroje przewodu pokarmowego zwierząt są podatne na inhibitujące działanie kwasów kaprylowego i kaprynowego dodawanych do kultur bakteryjnych w dość niskich stężeniach. Kwas oleinowy był znacznie mniej efektywny.