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Eva Skřivanová, Zuzana Molatová, Milan Marounek. Effects of caprylic acid and triacylglycerols of both caprylic and capric acid in rabbits experimentally infected with enteropathogenic O103. *Veterinary Microbiology*, 2009, 126 (4), pp.372. 10.1016/j.vetmic.2007.07.010 . hal-00532293

HAL Id: hal-00532293

<https://hal.science/hal-00532293>

Submitted on 4 Nov 2010

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Accepted Manuscript

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PII: S0378-1135(07)00338-0
DOI: doi:10.1016/j.vetmic.2007.07.010
Reference: VETMIC 3761

To appear in: *VETMIC*

Received date: 8-6-2007
Revised date: 9-7-2007
Accepted date: 10-7-2007

Please cite this article as: Skřivanová, E., Molatová, Z., Marounek, M., Effects of caprylic acid and triacylglycerols of both caprylic and capric acid in rabbits experimentally infected with enteropathogenic *Escherichia coli* O103, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.07.010

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1 Effects of caprylic acid and triacylglycerols of both caprylic and
2 capric acid in rabbits experimentally infected with enteropathogenic
3 *Escherichia coli* O103

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Abstract

Eighty-eight rabbits weaned at the age of 35 days were divided into 4 groups. Rabbits of the first two groups were fed a granulated feed, free of antimicrobials. Rabbits of the 3rd and the 4th group were fed the same diet, supplemented with caprylic acid at 5 g/kg, and with triacylglycerols (TAG) of caprylic and capric acid at 10 g/kg, respectively. Rabbits of the 2nd, 3rd and 4th group were challenged orally with 10⁹ cells of *Escherichia coli* of the O103 serogroup. Numbers of coliform bacteria in faeces of non-infected rabbits averaged 4.66 log₁₀ cfu/g. Six days after inoculation, caprylic acid and TAG of caprylic and capric acid decreased faecal output of coliforms from 10.18 ± 0.62 log₁₀ cfu/g to 7.79 ± 0.48 and 8.04 ± 0.50 log₁₀ cfu/g, respectively. In the 2nd, 3rd and 4th group 15, 11 and 9 infected rabbits died, respectively. However, the differences in mortality rate were not statistically significant. Surviving rabbits were slaughtered at 53 days of age. In caecal contents of infected rabbits, numbers of coliform bacteria were significantly reduced from 8.71 log₁₀ cfu/g to 5.55 – 5.83 log₁₀ cfu/g in treated groups. It can be concluded that both antimicrobial lipids are active against coliform bacteria, and may improve the resistance of weaned rabbits to enterocolitis.

Keywords: Rabbits; *Escherichia coli*; EPEC; Diarrhoea; Caprylic acid; Capric acid

1. Introduction

Diarrhoea is the major cause of high morbidity and mortality of young rabbits after weaning, and consequent economic losses, in commercial rabbitries. Enteropathogenic *Escherichia coli* (EPEC) strains of the O103 serogroup have frequently been identified as a causative agent of diarrheal disease (Blanco et al., 1996; D’Incau et al., 2006; Agnoletti et al., 2006). The pathogenicity of *E. coli* O103 strains in rabbits has been demonstrated experimentally (Camguilhem and Milon, 1990; Licois et al., 1992; Heczko et al., 2001; Tsalie et al., 2006). Gidenne and Licois (2005) reported that a high fibre intake improved the resistance of rabbits to the *E. coli* O103.

Several authors have shown that growth of *E. coli* was inhibited by medium-chain fatty acids containing 8 to 12 carbon atoms (Hassinen et al., 1951; Sprong et al., 2001; Marounek et al., 2003). In the presence of fatty acids the transport of protons through microbial membranes becomes uncontrolled. Consequently, fatty acids dissipate the electrochemical

proton gradient and disturb the energy metabolism in the cell (Galbraith and Miller, 1973). Caprylic acid (C₈) and triacylglycerols (TAG) of caprylic and capric acid (C₁₀) were tested in our previous experiments with rabbits (Skřivanová et al., 2002, 2006), however only effects on zootechnical parameters and digestibility coefficients were investigated. Therefore, the aim of the present study was to evaluate the effect of both additives on health status and shedding of *E. coli* in faeces of rabbits challenged with a strain of *E. coli* serotype O103.

2. Materials and methods

2.1. *E. coli* strain

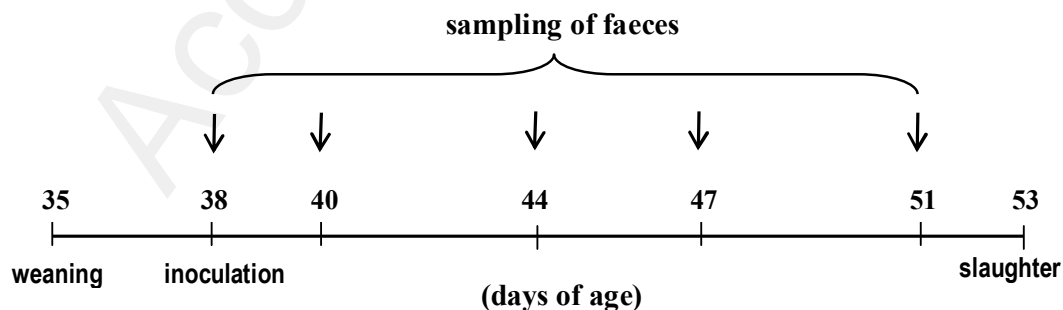
Strain E22 of *E. coli* O103:H2:K-, rhamnose negative, was kindly provided by Dr. A. Milon (National Veterinary College, Toulouse, France). The strain was isolated from the caecal content of a diarrheic rabbit (Camguilhem and Milon, 1989). *E. coli* E22 was grown in Wilkins-Chalgren broth (Oxoid) and maintained in 20% glycerol (v/v) at -70°C.

2.2. Animals diets, and challenge protocol

Eighty-eight Hyplus rabbits of both sexes, weaned at 35 days of age, were used in the experiment. Rabbits were healthy and free of coccidia. Animals were housed individually in 0.4 m x 0.5 m all-wire cages and randomly divided into four groups of 22 animals each. Rabbits of the 1st (live weight of 1061 ± 38 g) and the 2nd (live weight of 1130 ± 65 g) group were fed a granulated feed, free of antimicrobials, containing crude protein and crude fibre at 166 and 161 g/kg, respectively. Rabbits of the 3rd group (live weight of 1095 ± 35 g) were fed the same diet supplemented with caprylic acid (Sigma-Aldrich, Ltd, Prague, Czech Rep.) at 5 g/kg. Rabbits of the 4th group (live weight of 1076 ± 47 g) received the basal diet supplemented with Akomed R (Karlshamns, Sweden) at 10 g/kg. Akomed R is a commercially available oil, containing caprylic, capric and lauric acid at 60.8, 38.7 and 0.3 g per 100 g of fatty acids, respectively. Supplements were added at the expense of rapeseed oil in the basal diet. Cages of rabbits of the 1st group (negative control) were separated with a plastic barrier from other cages. The experiment was performed in accordance with the Czech

law no. 207/2004 Sb. and approved by the Ethical Committee of the Institute of Animal Science.

On the third day of the experiment rabbits of the 2nd, 3rd and 4th group were challenged orally with 0.5 ml of an overnight culture of *E. coli* E22 (ca 10⁹ bacteria per a rabbit). Rabbits were checked daily for morbidity and mortality, weighed individually once a week, and feed consumption was recorded daily. Two, six, nine and thirteen days after infection faecal samples of randomly selected rabbits (5 per each group) were taken for bacteriological analyses (see the experimental scheme below). Number of coliform bacteria was determined by a plating technique. Faecal samples were serially diluted with sterile saline and four dilutions from each sample plated on MacConkey agar (Oxoid) plates. After incubation (aerobically, at 37°C for 48 h) 10 typical colonies were isolated from each plate (4 plates per a group), and the proportion of *E. coli* O103 serotype was determined by the agglutination method (Salajka et al., 1992). Dead rabbits were examined using standard pathological, bacteriological and parasitological methods in the State Veterinary Institute (Prague). Survivors were slaughtered at 53 days of age, and gastric and caecal samples were taken. Serial dilutions were prepared and 0.1 ml aliquots were plated on MacConkey and Wilkins-Chalgren agar plates to determine counts of coliform bacteria and total anaerobic bacteria, respectively. Inoculated plates were incubated at 37°C for 48 h. Plates with MacConkey agar were incubated aerobically.



Data on mortality and the ratio of *E. coli* O103 to total coliforms were analyzed using the Fisher's exact test. To compare numbers of total anaerobic and coliform bacteria in control and infected rabbits, analysis of variance followed by Tukey's test was applied. All data were analyzed using the SAS programme (SAS, 2001).

3. Results

Forty-two of 66 infected rabbits suffered from diarrhoea and most of them died (Fig. 1). The mortality level was higher in the 2nd group, which was a positive control, than in rabbits receiving caprylic acid or Akomed R. Differences between groups of infected rabbits were not statistically significant ($P > 0.05$). Necropsy of dead rabbits revealed colibacillosis, in four rabbits accompanied with the occurrence of *Bordetella bronchiseptica* and *Pasteurella multocida*. Most rabbits were free of coccidia, in two rabbits sporadic occurrence of *Eimeria magna* was observed. The average daily gain of rabbits of the 1st group (non-infected rabbits) was 44.2 ± 4.6 g. Average daily gains of survivals of the 2nd, 3rd and the 4th group were 40.8 ± 6.6 g, 38.4 ± 7.3 g and 41.3 ± 5.9 g, respectively, with weight gains of rabbits of the 1st and the 3rd group significantly different ($P = 0.025$).

Numbers of coliform bacteria in faeces of non-infected rabbits varied from 4.13 to 5.28 log₁₀ cfu per g (Table 1). In faeces of infected rabbits numbers of coliform bacteria were significantly higher ($P < 0.001$), reaching the maximum at day 6 (group 3 and 4) or 9 (group 2) post inoculation. Caprylic acid and Akomed R significantly ($P < 0.001$) decreased faecal output of coliform bacteria. At the end of the experiment numbers of coliform bacteria in treated groups were decreased to values by four digit places lower than those in rabbits of the positive control. In faeces of non-inoculated rabbits bacteria belonging to the O103 serogroup were not detected. After inoculation, their proportion in total coliforms ranged from 67.5% to 95.0%.

After slaughter, numbers of coliform bacteria in the caecal content were significantly ($P < 0.01$) higher in infected than in non-infected rabbits (Table 2). Numbers of caecal

coliform bacteria were significantly ($P < 0.001$) reduced in rabbits fed diets supplemented with caprylic acid and Akomed R. Counts of total anaerobic bacteria were marginally, but significantly increased in all groups of infected rabbits.

4. Discussion

The present experiment confirmed the antimicrobial activity of caprylic acid and TAG of caprylic and capric acid added to the feed against EPEC in experimentally infected rabbits. In rabbits, caprylic and capric acid are synthesized *de novo* in the mammary gland and represent approximately one half of the total fatty acids in milk (Jones and Parker, 1981). Caprylic and capric acid thus contribute to a defence of sucklings against infection till weaning. Free medium-chain fatty acids, but not their TAG, are effective antimicrobial agents (Kabara et al., 1972). In the digestive tract of rabbits fatty acids are released from their ester bonds by gastric and pancreatic lipase. According to Skřivanová and Marounek (2002, 2006) both caprylic acid and TAG of caprylic and capric acid significantly decreased mortality of growing rabbits under practical field conditions, without affecting the rate of growth, feed intake and feed conversion. TAG of medium-chain fatty acids added to diets of piglets, together with lipolytic enzymes, reduced bacterial load in the upper part of the digestive tract and increased weight gain (Dierick et al., 2002). Caprylic acid and TAG of caprylic and capric acid thus may improve a non-specific defence of young rabbits after weaning against enteropathogenic bacteria.

Most of coliform bacteria in faeces of infected rabbits belonged to the inoculated *E.coli* O103 serotype. Its proportion in total coliforms was relatively constant in the course of the experiment (data not shown). This implies that the infection was accompanied by proliferation of other coliform bacteria. The kinetics of faecal shedding of coliform bacteria, mortality of infected rabbits, and the relative proportion of inoculated strain in total coliforms observed in this study were similar to that reported by Marchès et al. (2000), who used the same E22 strain of *E. coli* O103. Numbers of coliform bacteria in the caecum of non-

inoculated rabbits (2.5×10^4 cfu/g) were in agreement to those found by Camguilhem and
Milon (1990).

Conclusion

Caprylic acid and TAG of caprylic and capric acid significantly reduced numbers of
coliform bacteria in the caecum and faeces of rabbits experimentally infected with the
enteropathogenic strain of *E. coli* O103. Although mortality of infected rabbits was decreased
in treated groups, differences were non-significant. This could probably be attributed to a low
number of rabbits in control and experimental groups.

Acknowledgement

This study was supported by the Czech Science Foundation (project no. 523/07/P340).

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209 **Figure legend**

210 Fig. 1. Survival of rabbits from the 1st, 2nd, 3rd and 4th group: (○) negative control, (●) positive
211 control, (Δ) rabbits receiving the diet with 5 g/kg caprylic acid, and (▲) rabbits receiving the
212 diet with 10 g/kg Akomed R.

Table 1.

Mean numbers (\log_{10} cfu.g⁻¹) of coliform bacteria in faeces of rabbits before and after infection with *Escherichia coli* O103, strain E22

Treatment group	Diet	Infection	Before infection	Days after infection			
				2	6	9	13
1	Basal	No	4.70 ^a	5.28 ^a	5.24 ^a	4.79 ^a	4.50 ^a
2	Basal	Yes	4.21 ^a	7.53 ^b	10.18 ^b	10.53 ^b	9.68 ^b
3	Caprylic acid	Yes	4.47 ^a	6.44 ^b	7.79 ^c	7.33 ^c	5.70 ^c
4	Akomed R	Yes	4.13 ^a	6.21 ^{ab}	8.04 ^c	7.67 ^c	5.13 ^c
R.M.S.E.			0.29	0.60	0.56	0.56	0.68

R.M.S.E., Root mean square error.

^{abc} Values in the same column with different superscripts differ ($P < 0.05$).

Table 2.

Mean numbers (\log_{10} cfu.g⁻¹) of coliform and anerobic bacteria in contents of stomach and caecum of rabbits before and after infection with *Escherichia coli* O103, strain E22

Treatment group	Diet	Infection	Coliform bacteria		Anaerobic bacteria	
			Stomach	Caecum	Stomach	Caecum
1	Basal	No	< 2	4.39 ^a	3.94 ^a	8.44 ^a
2	Basal	Yes	< 2	8.71 ^b	5.46 ^b	9.81 ^b
3	Caprylic acid	Yes	< 2	5.83 ^c	3.73 ^a	9.50 ^b
4	Akomed R	Yes	< 2	5.55 ^c	3.96 ^a	9.37 ^b
R.M.S.E.			-	0.59	0.78	0.41

R.M.S.E., Root mean square error

^{ab} Values in the same column with different superscripts differ ($P < 0.05$)

