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Authors: Eva Skřivanová, Zuzana Molatová, Milan Marounek



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8 Abstract

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

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

22 1. Introduction

23 Diarrhoea is the major cause of high morbidity and mortality of young rabbits after
24 weaning, and consequent economic losses, in commercial rabbitries. Enteropathogenic
25 *Escherichia coli* (EPEC) strains of the O103 serogroup have frequently been identified as a
26 causative agent of diarrheal disease (Blanco et al., 1996; D’Incau et al., 2006; Agnoletti et al.,
27 2006). The pathogenicity of *E. coli* O103 strains in rabbits has been demonstrated
28 experimentally (Camguilhem and Milon, 1990; Licois et al., 1992; Heczko et al., 2001; Tsalie
29 et al., 2006). Gidenne and Licois (2005) reported that a high fibre intake improved the
30 resistance of rabbits to the *E. coli* O103.

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

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

41 **2. Materials and methods**

42 *2.1. E. coli strain*

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

47 *2.2. Animals diets, and challenge protocol*

48 Eighty-eight Hyplus rabbits of both sexes, weaned at 35 days of age, were used in the
49 experiment. Rabbits were healthy and free of coccidia. Animals were housed individually in
50 0.4 m x 0.5 m all-wire cages and randomly divided into four groups of 22 animals each.
51 Rabbits of the 1st (live weight of 1061 ± 38 g) and the 2nd (live weight of 1130 ± 65 g) group
52 were fed a granulated feed, free of antimicrobials, containing crude protein and crude fibre at
53 166 and 161 g/kg, respectively. Rabbits of the 3rd group (live weight of 1095 ± 35 g) were fed
54 the same diet supplemented with caprylic acid (Sigma-Aldrich, Ltd, Prague, Czech Rep.) at 5
55 g/kg. Rabbits of the 4th group (live weight of 1076 ± 47 g) received the basal diet
56 supplemented with Akomed R (Karlshamns, Sweden) at 10 g/kg. Akomed R is a
57 commercially available oil, containing caprylic, capric and lauric acid at 60.8, 38.7 and 0.3 g
58 per 100 g of fatty acids, respectively. Supplements were added at the expense of rapeseed oil
59 in the basal diet. Cages of rabbits of the 1st group (negative control) were separated with a
60 plastic barrier from other cages. The experiment was performed in accordance with the Czech

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

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

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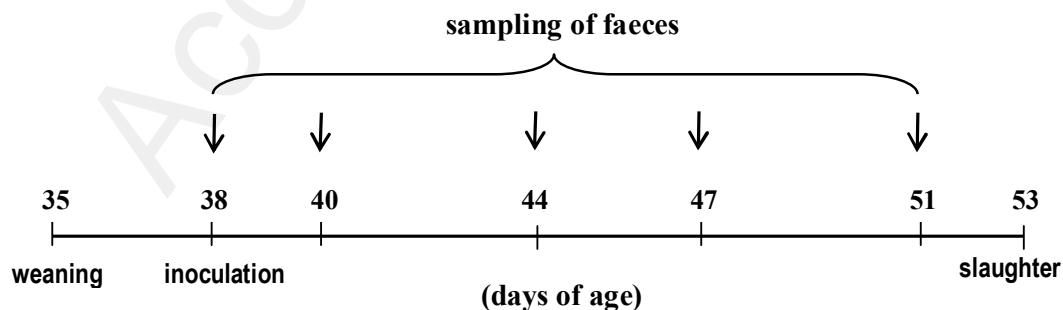
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86 Data on mortality and the ratio of *E. coli* O103 to total coliforms were analyzed using the
87 Fisher's exact test. To compare numbers of total anaerobic and coliform bacteria in control
88 and infected rabbits, analysis of variance followed by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

90 3. Results

91 Forty-two of 66 infected rabbits suffered from diarrhoea and most of them died (Fig. 1).
92 The mortality level was higher in the 2nd group, which was a positive control, than in rabbits
93 receiving caprylic acid or Akomed R. Differences between groups of infected rabbits were not
94 statistically significant ($P > 0.05$). Necropsy of dead rabbits revealed colibacillosis, in four
95 rabbits accompanied with the occurrence of *Bordetella bronchiseptica* and *Pasteurella*
96 *multocida*. Most rabbits were free of coccidia, in two rabbits sporadic occurrence of *Eimeria*
97 *magna* was observed. The average daily gain of rabbits of the 1st group (non-infected rabbits)
98 was 44.2 ± 4.6 g. Average daily gains of survivals of the 2nd, 3rd and the 4th group were $40.8 \pm$
99 6.6 g, 38.4 ± 7.3 g and 41.3 ± 5.9 g, respectively, with weight gains of rabbits of the 1st and
100 the 3rd group significantly different ($P = 0.025$).

101 Numbers of coliform bacteria in faeces of non-infected rabbits varied from 4.13 to 5.28
102 \log_{10} cfu per g (Table 1). In faeces of infected rabbits numbers of coliform bacteria were
103 significantly higher ($P < 0.001$), reaching the maximum at day 6 (group 3 and 4) or 9 (group
104 2) post inoculation. Caprylic acid and Akomed R significantly ($P < 0.001$) decreased faecal
105 output of coliform bacteria. At the end of the experiment numbers of coliform bacteria in
106 treated groups were decreased to values by four digit places lower than those in rabbits of the
107 positive control. In faeces of non-inoculated rabbits bacteria belonging to the O103 serogroup
108 were not detected. After inoculation, their proportion in total coliforms ranged from 67.5% to
109 95.0%.

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

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

115 4. Discussion

116 The present experiment confirmed the antimicrobial activity of caprylic acid and TAG of
117 caprylic and capric acid added to the feed against EPEC in experimentally infected rabbits. In
118 rabbits, caprylic and capric acid are synthesized *de novo* in the mammary gland and represent
119 approximately one half of the total fatty acids in milk (Jones and Parker, 1981). Caprylic and
120 capric acid thus contribute to a defence of sucklings against infection till weaning. Free
121 medium-chain fatty acids, but not their TAG, are effective antimicrobial agents (Kabara et al.,
122 1972). In the digestive tract of rabbits fatty acids are released from their ester bonds by gastric
123 and pancreatic lipase. According to Skřivanová and Marounek (2002, 2006) both caprylic
124 acid and TAG of caprylic and capric acid significantly decreased mortality of growing rabbits
125 under practical field conditions, without affecting the rate of growth, feed intake and feed
126 conversion. TAG of medium-chain fatty acids added to diets of piglets, together with lipolytic
127 enzymes, reduced bacterial load in the upper part of the digestive tract and increased weight
128 gain (Dierick et al., 2002). Caprylic acid and TAG of caprylic and capric acid thus may
129 improve a non-specific defence of young rabbits after weaning against enteropathogenic
130 bacteria.

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

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

140 **Conclusion**

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

146 **Acknowledgement**

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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.

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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 anaerobic 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 ($P < 0.05$)

