A NOTE ON THE EFFECT OF CAPRYLIC ACID ON SHEDDING OF *ESCHERICHIA COLI* O103 AND O128 IN WEANED RABBITS

Skřivanová E.*, Marounek M., Skřivanová V.

Institute of Animal Production, Přátelství 815, 10401 Prague, Czech Republic
*Corresponding author: skrivanova.eva@vuzv.cz

ABSTRACT

Enteropathogenic *Escherichia coli* (EPEC) colibacillosis represents one of the major causes of enteritis in rabbit breedings. The aim of the present study was to evaluate the effect of caprylic acid and triacylglycerols of caprylic, capric and lauric acid on health status and shedding of *E. coli* in faeces of rabbits infected with EPEC O103 or O128. Two experiments were carried out. In each experiment, fourty-four Hyplus rabbits, weaned at 35 days of age were used. Rabbits were housed individually and randomly divided into four groups of 12 animals. Rabbits of the 1st and the 2nd group were fed a granulated feed, free of antimicrobials. Rabbits of the 3rd group were fed the same diet supplemented with caprylic acid at 5 g/kg. Rabbits of the 4th group received the basal diet supplemented with commercially available oil (Akomed R), containing caprylic, capric and lauric acid at 60.8, 38.7 and 0.3 g/100 g of fatty acids, respectively. Supplements were added at expense of rapeseed oil in the basal diet. In the first experiment, the feed of rabbits of the 2nd, 3rd and 4th group was contaminated with 0.5 ml of an overnight culture of *E. coli* O103. In the second experiment, *E. coli* O128 was used. Rabbits were checked daily for morbidity and mortality. Faecal shedding of *E. coli* was examined three times a week. After the incubation, 10 typical colonies were isolated from each plate and detection of *E. coli* O103 or O128 serotype was determined. Weight of rabbits and feed consumption were recorded. Survived rabbits were slaughtered on the 55th day of the experiment. Immediately after the slaughter, gastric and caecal contents were taken to determine counts of *E. coli* and total anaerobic bacteria by plating technique. Numbers of *E. coli* in faeces of non-infected rabbits varied from 3.24 to 4.10 $\log_{10}$ cfu/g. In both experiments, caprylic acid and Akomed R significantly decreased faecal output of *E. coli* ($P<0.05$). Ten days after inoculation, corresponding numbers were 10.13 $\log_{10}$ cfu/g (positive control), 5.99 $\log_{10}$ cfu/g (caprylic acid), and 8.31 $\log_{10}$ cfu/g (Akomed R) in the first experiment. In the second experiment, *E. coli* shedding decreased from 10.49 $\log_{10}$ cfu/g in positive control, to 5.99 $\log_{10}$ cfu/g (caprylic acid) and 6.90 $\log_{10}$ cfu/g (Akomed R) on the 9th day of the experiment. In faeces of non-inoculated rabbits, bacteria belonging to the O103 or O128 serogroup were not detected. In both experiments, numbers of caecal *E. coli* were significantly reduced in rabbits fed diets supplemented with caprylic acid and Akomed R, and were not statistically different from numbers of *E. coli* in the negative control ($P>0.05$). It can be concluded that both caprylic acid and triacylglycerols of caprylic and capric acid are effective in the reduction of EPEC in the caecum and faeces of infected rabbits.

Key words: Caprylic acid, Enteropathogenic, *Escherichia coli*, Infection.

INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) is one of the major pathogens causing enteritis in rabbits (Camguilhem and Milon, 1989; Blanco *et al*., 1996). In weaned rabbits, serotypes O15, O26 and O103 are the most relevant, whereas serotypes O128 and O132 are of variable virulence (Peeters *et al*., 1988). In France, Spain and Italy, the disease is mainly associated with strains of serogroup O103 (Camguilhem and Milon, 1989; Bohez *et al.,* 2004). In Belgium and Netherlands, *E. coli* O15 represents the most predominant serotype (Bohez *et al*., 2004). Presumably in other countries these *E. coli* serotypes belong to important rabbit pathogens as well. High mortality and morbidity of young rabbits infected with this pathogen leads to consequent economic losses in farms.

Protection against this pathogen is a challenging goal not only in rabbits, but also in other mammal species. Fatty acids and their derivatives proved strong antibacterial activity in numbers of investigations using various bacteria. The target microorganisms have included both Gram-positive and Gram-negative bacteria, including major food-borne pathogens. Several authors have shown that growth of *E. coli* was inhibited by medium-chain fatty acids containing 8-12 carbon atoms (Hassinen *et al*., 1951; Sprong *et al*., 2001; Marounek *et al*., 2003). The effect of caprylic acid and triacylglycerols of caprylic, capric and lauric acid was tested in our previous experiment (Skřivanová *et al*., 2007), where the infection with *E. coli* O103 *per os* was induced. 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 infected with *E. coli* O103 or O128. Infection was induced via contaminated feed.

MATERIALS AND METHODS

E. coli strains

Strain E22 of *E. coli* O103:H2:K-, rhamnose negative, and strain C6 of *E. coli* O128, rhamnose negative, were kindly provided by Dr. A. Milon (National Veterinary College, Toulouse, France). Both strains were isolated from the caecal contents of diarrheic rabbits (Camguilhem and Milon, 1989). *E. coli* E22 and C6 were grown in Wilkins-Chalgren broth (Oxoid, Czech Republic) and maintained in 20% glycerol (v/v) at -70°C.

Animals diets

Two experiments were carried out. In each experiment, fourty-four Hyplus rabbits of both sexes, weaned at 35 days of age were used. Rabbits were housed individually and randomly divided into four groups of 12 animals. Rabbits of the 1st and the 2nd 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 were fed the same diet supplemented with caprylic acid (Sigma-Aldrich, Ltd, Czech Republic) at 5 g/kg. Rabbits of the 4th group received the basal diet supplemented with Akomed R (Karlshamns, Sweden) at 10 g/kg. Akomed R is 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 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.

1st experiment

On the third day of the experiment, the feed of rabbits belonging to the 2nd, 3rd and 4th group was contaminated with 0.5 ml of an overnight culture of *E. coli* O103 (ca 10^9 bacteria per a rabbit). Rabbits were checked daily for morbidity and mortality. Weights of rabbits were recorded once a week. Feed consumption was recorded daily. Two, five, seven, ten and thirteen days after infection, faecal samples of randomly selected rabbits (five rabbits per each group) were taken for bacteriological analyses. Numbers of *E. coli* were determined by plating technique on TBX plates (Oxoid, Czech Republic). After incubation (aerobically, at 37°C for 24 h), 10 typical colonies were isolated from each plate (4 plates per a group) and detection of *E. coli* O103 serotype was determined (DrySpot Serocheck, Oxoid, Czech Republic).

2nd experiment

Challenge protocol of the second experiment was similar, with minor changes: rabbits were challenged with *E. coli* O128 and samples for bacteriological analyses were taken on the second, fifth, seventh, nineth, twelveth and fourteenth day after the infection.

Rabbits that died during both experiments were examined using standard pathological, bacteriological and parasitological methods in the State Veterinary Institute (Prague). Survivors were slaughtered at

55th day of age. Immediately after the slaughter, gastric and caecal contents were removed and samples taken to determine counts of *E. coli* and total anaerobic bacteria by plating on TBX and Wilkins-Chalgren agar plates (Oxoid, Czech Republic), respectively. Inoculated plates were incubated at 37°C for 48 h. Plates with Wilkins-Chalgren agar were incubated anaerobically.

Data on mortality and on ratio of *E. coli* O103 or O128 to total numbers of tested colonies 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).

RESULTS AND DISCUSSION

1st experiment

Twenty-eight out of 36 infected rabbits suffered from diarrhoea. The mortality level was higher in the 2nd group (91.7%), which was a positive control, than in rabbits receiving caprylic acid (66.7%) or Akomed R (75%). Differences between groups of infected rabbits were not statistically significant. Necropsy of dead rabbits revealed colibacillosis, in some cases accompanied with the occurrence of *Pasteurella multocida* (3 cases). Most rabbits were free of coccidia, in three cases, sporadic occurrence of *Eimeria magna* was observed.

Numbers of *E. coli* in faeces of non-infected rabbits varied from 3.24 to 3.49 $\log_{10}$ cfu per g (Table 1). There were no differences between groups observed prior to the infection (P>0.05). Shedding of *E. coli* in rabbits belonging to the positive control (2nd group) was significantly higher than in treated groups in the course of the experiment. Caprylic acid and Akomed R significantly decreased faecal output of *E. coli*. Ten days after inoculation, corresponding numbers were 10.13 $\log_{10}$ cfu per g (positive control), 5.99 $\log_{10}$ cfu per g (caprylic acid), and 8.31 $\log_{10}$ cfu per g (Akomed R). In faeces of non-inoculated rabbits, bacteria belonging to the O103 serogroup were not detected (data not shown).

Table 1: Mean numbers ($\log_{10}$ CFU/g) of *E. coli* in faeces of rabbits before and after infection with *E. coli* O103, strain E22

Treatment group	Diet	Infection	Before infection	Days after infection				
				3	5	7	10	13
1	Basal	No	3.27	4.07^{a}	4.12^{a}	4.59^{a}	4.78^{a}	4.18^{a}
2	Basal	Yes	3.36	9.57^{b}	10.00^{b}	9.51^{b}	10.13^{b}	10.33^{b}
3	Caprylic acid	Yes	3.24	8.93^{c}	8.06^{c}	7.82^{c}	5.99^{a}	6.80^{c}
4	Akomed R	Yes	3.49	7.63^{c}	8.18^{c}	8.98^{bc}	8.31^{c}	8.15^{c}
RMSE			0.33	0.60	0.55	0.44	0.46	0.44

RMSE: Root mean square error; $^{a, b, c}$Values in the same column with the different superscript differ significantly (P<0.05)

The kinetics of faecal shedding of *E. coli* observed in this study was similar to that reported by Marches *et al*. (2000), who used the same E22 strain of *E. coli* O103. Numbers of *E. coli* in the caecum of non-inoculated rabbits were in agreement with those found by Camguilhem and Milon (1990). Results in the present study were comparable to that observed in our previous experiment (Skřivanová *et al*., 2007), where the infection of rabbits was induced *per os*.

After the slaughter, numbers of *E. coli* in the caecal content averaged 4.77 $\log_{10}$ cfu per g in non-infected rabbits, and 9.07 $\log_{10}$ cfu per g in infected rabbits of the 2nd group (Table 2). Numbers of caecal *E. coli* were significantly reduced in rabbits fed diets supplemented with caprylic acid and Akomed R and were not statistically different from numbers of *E. coli* in the negative control (P>0.05).

Table 2: Mean numbers (log_{10} CFU/g) of *E. coli* and anaerobic bacteria in contents of stomach and caecum of rabbits infected with *E. coli* O103, strain E22

Treatment group	Diet	Infection	*E. coli*		Anaerobic bacteria	
			Stomach	Caecum	Stomach	Caecum
1	Basal	No	2.41	4.77[a]	4.63	9.16
2	Basal	Yes	2.57	9.07[b]	4.45	9.18
3	Caprylic acid	Yes	2.30	4.80[a]	4.41	8.61
4	Akomed R	Yes	2.63	6.33[a]	5.03	9.20
RMSE			0.68	0.45	0.49	0.60

RMSE: Root mean square error; [a, b, c]Values in the same column with the different superscript differ significantly (P<0.05)

2nd experiment

The course of the infection with *E. coli* O128 was more moderate. The addition of the serotype O128 to the feed caused only mild diarrhoea in six rabbits from the 2nd group, two of these animals died. Only in one rabbit from the group receiving caprylic acid and one rabbit from the group receiving Akomed R, mild diarrhoea occured. No rabbit from the 1st, 3rd and the 4th group died. Differences in mortality and morbidity between experimental groups were not statistically significant. Necropsy of dead rabbits revealed colibacillosis.

E. coli shedding decreased significantly in both treated groups (Table 3). Numbers of caecal *E. coli* were significantly reduced in rabbits fed diets supplemented with caprylic acid and Akomed R and were not statistically different from numbers of *E. coli* in the negative control (P>0.05).

Table 3: Mean numbers (log_{10} CFU/g) of *E. coli* in faeces of rabbits before and after infection with *E. coli* O128, strain C6

Treatment group	Diet	Infection	Before infection	Days after infection					
				2	5	7	9	12	14
1	Basal	No	4.01	4.32[a]	4.23[a]	4.14[a]	4.31[a]	3.81[a]	3.91[b]
2	Basal	Yes	4.10	6.36[b]	9.62[b]	10.22[b]	10.49[b]	9.38[b]	7.97[a]
3	Caprylic acid	Yes	3.77	4.90[ab]	6.10[c]	5.78[c]	5.99[c]	5.78[c]	4.91[b]
4	Akomed R	Yes	4.00	5.57[ab]	7.33[c]	6.06[c]	6.90[c]	6.69[c]	5.12[b]
RMSE			0.56	0.81	0.67	0.68	0.62	0.70	0.82

RMSE: Root mean square error; [a, b, c]Values in the same column with the different superscript differ significantly (P<0.05)

Table 4: Mean numbers (log_{10} CFU/g) of *E. coli* and anaerobic bacteria in contents of stomach and caecum of rabbits infected with *E. coli* O128, strain C6

Treatment group	Diet	Infection	*E. coli*		Anaerobic bacteria	
			Stomach	Caecum	Stomach	Caecum
1	Basal	No	2.05	3.82[a]	3.72	9.08
2	Basal	Yes	2.08	7.53[b]	4.85	9.55
3	Caprylic acid	Yes	2.03	4.13[a]	3.53	9.48
4	Akomed R	Yes	2.19	4.93[a]	3.77	9.45
RMSE			0.21	0.74	0.77	0.42

RMSE: Root mean square error; [a, b, c]Values in the same column with the different superscript differ significantly (P<0.05)

Present results confirmed the reduction of the colonisation of the gut caused by caprylic acid and triacylglycerols of caprylic and capric acid added to the feed of experimentally infected rabbits. In rabbits, caprylic and capric acid represent approximately one half of the total fatty acids in milk (Jones and Parker, 1981). Gallois *et al*. (2007) observed that suckling protects young rabbits from lethal colibacillosis and delays the evolution of diarrhea. Our results support the theory that caprylic and capric acid present in rabbit milk play important role in the defence of suckling rabbits against the infection during weaning.

CONCLUSIONS

Both caprylic acid and triacylglycerols of caprylic and capric acid are effective in the reduction of EPEC in the caecum and faeces of rabbits infected with EPEC O103 or O128.

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REFERENCES

Blanco J.E., Blanco M., Blanco J., Mora A., Balaguer L., Mourino M., Juarez A., Jansen W.H. 1996. O serogroups, biotypes, and *eae* genes in *Escherichia coli* strains isolated from diarrheic and healthy rabbits. *J. Clin. Microbiol., 34, 3101-3107.*

Bohez L., Maertens L., Laevens H., Stakenborg T., Peeters J., Vandekerchove D. 2004. *In: Proc. 8th World Rabbit Congress, Puebla, Mexico, 439-444.*

Camguilhem R., Milon A. 1989. Biotypes and O serogroups of Escherichia coli involved in intestinal infections of weaned rabbits: clues to diagnosis of pathogenic strains. *J. Clin. Microbiol., 27, 743-747.*

Camguilhem R., Milon A. 1990. Protection of weaned rabbits against experimental *Escherichia coli* O103 intestinal infection by oral formalin-killed vaccine. *Vet. Microbiol., 21, 353-362.*

Gallois M., Gidenne T., Tasca Ch., Caubet C., Coudert C., Milon A., Boullier S. 2007. Maternal milk contains antimicrobial factors that protects young rabbits from enteropathogenic *Escherichia coli* infection. *Clin. Vaccine Immunol., 14, 585-592.*

Hassinen J.B., Durbin G.T., Bernhart F.W. 1951. The bacteriostatic effect of saturated fatty acids. *Arch. Biochem. Biophys., 31, 183-189.*

Jones C.S., Parker D.S. 1981. The metabolism of glucose, acetate and palmitate in the lactating rabbit. *Comp. Biochem. Physiol., B69, 837-842.*

Marches O., Nougayrede J.P., Boullier S., Mainil J., Charlier G., Raymond I., Pohl P., Boury M., De Rycke J., Milon A., Oswald E. 2000. Role of tir and intimin in the virulence of rabbit enteropathogenic *Escherichia coli* serotype O103:H2. *Infect. Immun., 68, 2171-2182.*

Marounek M., Skřivanová E., Rada V. 2003. Susceptibility of *Escherichia coli* to C_2-C_{18} fatty acids. *Folia Microbiol., 48, 731-735.*

Peeters J.E., Geeroms R., Orskov F. 1988. Biotype, serotype, and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated form diarrheic commercial rabbits. *Infect. Immun., 56, 1442-1448.*

Skřivanová E., Molatová Z., Marounek M. 2007. Effects of caprylic acid and triacylglycerols of both caprylic and capric acid on rabbits experimentally infected with enteropathogenic *Escherichia coli* O103. *Vet. Microbiol. Doi: 10.1016/j.vetmic.2007.07.010.*

Sprong R.C., Hulstein M.F.E., Van der Meer R. 2001. Bactericidal activities of milk lipids. *Antimicrob. Agents Chemother., 45, 1298-1301.*