EFFECT OF ADDING DIETARY CAPRYLIC ACID ON THE BACTERIAL POPULATION IN THE RABBIT CAECUM AND STOMACH

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ABSTRACT

The effect of dietary supplementation with caprylic acid on the bacterial population of the rabbit caecum and stomach was investigated using two different PCR-based methods: Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal Restriction Fragment Length Polymorphisms (TRFLP). Caprylic acid was added to the diet either in the form of 5 g/kg of the pure acid, or within Akomed R at 10 g/kg (with or without lipase at 10 g/kg). Rabbits fed ad libitum on their respective diets for 27 days before being euthanized and the digestive contents of the caecum and stomach removed for DNA analysis.

Neither analytical method suggested a change in the bacterial populations in the stomach dependent on the feeding group of the animal (control, caprylic acid, Akomed R or Akomed R and lipase supplementation). However, the use of DGGE suggested that the caecal samples could be split into two groups; those with no additional fatty acid supplementation of the diet, or where lipase was added along with the fatty acids; and the other where there was caprylic acid added either in its pure form, or in conjunction with other fatty acids in the form of Akomed R supplementation. Furthermore, there was an apparent increase in the detectable biodiversity following supplementation with either caprylic acid or Akomed R. No such segregation between dietary regimes was detected by TRFLP analysis. This is suggestive of there being a shift in relative numbers of specific organisms (DGGE data) without this effect being restricted specifically to one large-scale taxonomic group of organisms (TRFLP data). Thus dietary supplementation with caprylic acid, either in a pure form, or in conjunction with other medium-chain fatty acids, has the ability to target specific groups of microbes, whilst allowing a relatively large-scale bacterial diversity to persist.

Key words: Bacterial populations, Stomach, Caecum, Rabbit, Caprylic acid.

INTRODUCTION

The antimicrobial activity of medium-chain fatty acids (MCFA), containing 8 to 14 carbon atoms, and their monoglycerides has been studied extensively in recent years (reviewed by Thormar and Bergsson, 2001). However, the mode of action of MCFA in the animal digestive tract is not fully understood. Smith (1965) found the stomach and small intestinal contents of weaned rabbits were almost sterile. It was suggested that this was due to the rabbit milk fat containing antimicrobial compounds, identified as caprylic and capric acid (Canas-Rodriguez and Smith, 1981). A protective role of rabbit milk was demonstrated in rabbits experimentally infected with enteropathogenic E. coli – EPEC – (Gallois et al., 2007). Caprylic acid had no effect on the growth rate of rabbits, but decreased mortality in the post-weaning period (Skřivanová and Marounek, 2002). Supplementation of the rabbit diet with caprylic acid and triacylglycerols of caprylic and capric acid decreased bacterial shedding in rabbits experimentally infected with EPEC O103 (Skřivan et al., 2005; Skřivanová et al., 2007).
The aim of the present study was to evaluate if the supplementation of rabbit diet with MCFA affects bacterial diversity in the stomach and caecum of weaned rabbits.

**MATERIALS AND METHODS**

**Animals and experimental design**

Twenty weaned Hyplus rabbits of both sexes, 30 days of age, with initial weights of $771 \pm 31 \text{ g}$ were purchased from a commercial rabbitry (Dvory, Czech Republic). All nutritional experimentation was carried out at the Institute of Animal Science (Prague, Czech Republic).

Animals were randomly divided into four groups and housed in individual metabolic cages in an environmentally controlled stable. Rabbits in Group 1 were fed a commonly-used commercial mixed feed. Group 2 received the diet supplemented with caprylic acid (Sigma-Aldrich) at 5 g/kg. Rabbits in Group 3 were given basal diet supplemented with Akomed R (Karlshamns, Sweden) at 10 g/kg. Group 4 received the diet supplemented with Akomed R (10 g/kg) together with lipase (Texazym PES) at 10 g/kg. Caprylic acid and Akomed R supplementation was compensated for by rapeseed oil in the basal diet. All diets were antibiotic-free, supplemented with salinomycin, and pelleted. Rabbits were given diets *ad libitum* for 27 days, and then slaughtered.

**Table 1:** Ingredients and determined chemical composition of the basal rabbit diet

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Chemical composition (%)</th>
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<tbody>
<tr>
<td>Alfalfa meal</td>
<td>Dry matter 91.1</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>Crude protein 17.7</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>Fat 3.8</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Ash 8.0</td>
</tr>
<tr>
<td>Sugar beet pulp</td>
<td>Starch 15.9</td>
</tr>
<tr>
<td>Oats</td>
<td>NDF 40.1</td>
</tr>
<tr>
<td>Barley</td>
<td>ADF 18.0</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>ADL 3.4</td>
</tr>
<tr>
<td>Vitamin supplement$^1$</td>
<td>1</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.5</td>
</tr>
<tr>
<td>Lime stone</td>
<td>1</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^1$Per kg of supplement: Vitamin A: 1 200 000 IU, vitamin D$_3$: 200 000 IU, vitamin E: 5 g, vitamin K$_1$: 0.2 g, vitamin B$_6$: 0.3 g, vitamin B$_{12}$: 0.7 g, vitamin B$_2$: 0.4 g, niacinamide: 5 g, Ca-pantothenate: 2 g, folic acid: 0.17 g, biotin: 20 mg, vitamin B$_1$: 2 mg, choline: 60 g, lysine: 25 g, DL-methionine: 100 g, salinomycin: 2.25 g.

**Molecular Analyses**

Immediately after slaughter samples of stomach and caecal contents were removed and stored at -70°C until analysis by molecular techniques. Digesta was broken up by bead beating. The sample was beaten for 30 seconds at 50 x 100 rpm (maximum speed) in a Minibeadbeater™ (Biospec products Inc.). Two tubes were beaten for each sample, pooled and stored at -20°C. Following bead beating, DNA was extracted from the cells using the QIAGEN QIAamp® DNA stool mini kits (Qiagen Ltd., UK). The manufacturer’s methodology was adhered to other detailed below. Firstly, samples were incubated at 95°C for 5 minutes to lyse all bacteria, including the Gram-positive bacteria (manufacturer's guidelines), and to maximise DNA retrieval yields. Samples were centrifuged at 13,000 g in a Minispin (Eppendorf AG) for 2 minutes, as this was found to improve the residue deposition which facilitated extraction. After lysis, DNA damaging substances and PCR inhibitors were removed by absorption to InhibitEX (QIAGEN). The supernatant was extracted following centrifugation (13,000 g for 3 minutes). The DNA purification methodology also included the addition of proteinase K, which digested any proteins in the supernatant. Binding DNA to a QIAamp spin column facilitated the removal of impurities and retrieval of pure DNA.

PCR was performed using “DGGE” bacterial primers 5’-CGC CCG CCG CGC GCG GCG GGG GCG GGG CCA CGG GGG GCC TAC GGG AGG CAG–3’ and 5’-ATT ACC GCG
DGGE analysis of the bacterial population of the samples from the stomach (Figure 1A) did not reveal any population differences. However, there was a split in the samples from the caecal population (Figure 1B), into two general clusters: one with no additional fatty acid supplementation of the diet, or where lipase had been added; and the other where there was caprylic acid in its pure form, or in conjunction with other fatty acids in the form of Akomed R supplementation. It is interesting to note that the branch lengths in these dendograms are proportional to differences between the samples. Thus, there is an increase in the detectable biodiversity following supplementation with either caprylic acid or Akomed R. It is important to remember that this is not an increase in the absolute diversity, but rather in the detectable diversity. This is presumably due to the survival of some of the species which are present at a minor level, at the expense of some of those which had been generally more abundant.
Using an alternative analysis, TRFLP, there was still no clustering pattern observed with samples from the stomach (Figure 2A). However, the caecal clustering effect with DGGE (Figure 1B) was not seen with TRFLP (Figure 2B). At first the DGGE and TRFLP results appear to conflict. However, it is important to consider the basis of the two techniques. DGGE relies on dissociating double stranded DNA as it moves through a gradient of increasing denaturant. This is what permits resolution between two fragments which differ by a single nucleotide. In relatively simple ecosystems, this implies that each band on the gel corresponds to a single DNA fragment. In a more complex ecosystem, such as the digestive tract, there are multiple sequences co-migrating within what appears to be a single band. Thus, different sequences of DNA with similar denaturing properties will appear to have similar mobilities on the denaturing gradient. This is already known, with multiple sequences being isolated from apparently single bands (e.g. Abecia et al., 2007). Conversely, strands which co-migrate on the TRFLP columns share 5’ terminal restriction fragment lengths and it is likely, although not implicit, that many of the co-migrating fragments are likely to have come from biologically related sources. This assumption is based on the premise that the probability of acquiring or losing a restriction cutting site in a particular piece of DNA is a relatively rare event. Thus, the two analytical methods are actually identifying two different things, with DGGE acting as a general indicator of changes in the population, and TRFLP acting as an indicator of changes between populations, by taking taxonomic relationship into consideration. Hence, there is a change in the population of bacteria based on the profiles determined by DGGE. However, it is interesting to note that the data generated by TRFLP imply that this is not something which can be attributed to the disappearance of a particular taxonomic group – based on the assumption that terminal fragment lengths are going to be conserved within a number, although not all, of closely related organisms.
CONCLUSIONS

Dietary supplementation with caprylic acid, either in a pure form, or in conjunction with other medium-chain fatty acids, has the ability to target specific groups of microbes, whilst allowing a relatively large-scale bacterial diversity to persist in the caecum.

REFERENCES


