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MICROFLORA OF RABBITS AFTER QUERCETIN AND T - 2 TOXIN APPLICATIONS

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Abstract

The aim of this study was to compare the microbial species in gut microflora of rabbit in control group against experimental group where it was applied quercetin and T2 toxin. It is the first study about caecum microflora study after quercetin and T2 toxin application. In this study for enumeration of bacteria we used classical methods. Enterococci were counted on Slanetz-Bartley agar (Biolife, Italy) and incubated at 37 °C for 48-72 hours, lactobacilli were counted on MRS Lactobacillus agar (Biolife, Italy) and incubated at 37 °C for 48-72 hours and coliform bacteria were counted on MacConkey agar (Biolife, Italy) and incubated at 37 °C for 24-48 hours. In our study the number of coliform bacteria ranged between 4.23 to 5.50 log CFU.g⁻¹, lactobacilli ranged between 3.20 to 4.54 log CFU.g⁻¹ and enterococci ranged between 3.28 to 5.23 log CFU.g⁻¹.

Keywords: microflora, rabbits, quercetin, T2 toxin

Introduction

Rabbits are monogastric herbivores, which are widely used for research purposes and husbandry. In rabbits, most food transformation events occur in the caecum, which is densely populated with bacteria and constitute an efficient continuous culture system. Rabbits help to maintain a relatively stable microbial population by caecotrophy. Notwithstanding this, relatively little attention has been paid to the microbial populations of the rabbit gut. Most studies have focused on the anaerobic microbiota of the caecum (Crociani *et al.* 1984; Forsythe and Parker, 1985) and intestinal archaea (Lelkes and Chang, 1987; Straw, 1988; Milon, 1996). These studies have revealed that the intestinal microbiota of rabbits consists predominantly of strictly anaerobic, Gram-negative bacteria belonging to the genus *Bacteroides*, while anaerobic sporulated Gram-positive bacteria are present in significantly lower numbers (Forsythe and Parker, 1985; Zomborszky-Kovacs *et al.* 2000). Facultative anaerobic bacteria isolated from the intestinal tract of rabbits belong to the Gram-positive genera *Bacillus*, *Enterococcus* and *Staphylococcus*, and Gram-negative *Enterobacter* and *Escherichia* (Forsythe and Parker, 1985; Canganella *et al.*, 1992). In contrast with other mammals, lactobacilli are very rarely found (Yu and Tsen, 1993).

Probiotics along with other functional foods positively affect the health of the consumer (Horská, 2012). Flavonoids have high antioxidant activities as free radical scavengers and as inhibitors of enzymes generating reactive oxygen species (Hollman and Katan, 1999). Flavonoids may preserve β -cell function by reducing oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes. In fact, quercetin, the main dietary flavonol, prevents and protects streptozotocin-induced oxidative stress and β -cell damage in rat pancreas (Coskun *et al.*, 2005) and decreased blood glucose concentration in both alloxan- and streptozotocin-induced diabetic rats (Coskun *et al.*, 2005; Romero *et al.*, 2010). Moreover, the intake of some specific types of flavonoids, including quercetin, was inversely associated with a higher risk of incident type 2 diabetes (Knekt *et al.*, 2002). Mycotoxins represent one of the most important categories of biologically produced natural toxins relative to human health and economic impact worldwide (Cetin and Bullerman, 2005; McKean *et al.*, 2006). T-2 is a genotoxic and cytotoxic mycotoxin, which produces inhibition of protein synthesis by strong affinity for the 60S ribosomal unit

and inhibition of RNA and DNA synthesis. Moreover, it can induce mutation and apoptosis. All these effects were observed both *in vivo* and *in vitro* (Bouaziz *et al.*, 2008; Chaudhari *et al.*, 2009).

The purpose of this study was to compare the microbial species in gut microflora of rabbit in control group against experimental group where it was applied quercetin and T2 toxin. It is first study about caecum microflora study after quercetin and T2 toxin application. In this study for enumeration of bacteria were using classical method.

Material and methods

Animals and diet, blood collection and determination of the parameters

Animals and diet

Adult female rabbits (n = 20) and male rabbits (n = 20) of meat line M91, maternal albinotic line (crossbreed Newzealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in experiment. Rabbits were healthy and their condition was judged as good at the commencement of the experiment. Water was available at any time from automatic drinking troughs. Groups of adult animals were balanced for age (150 days) and body weight (4 ± 0.5 kg) at the beginning of the experiment. Adult rabbits were fed diet of a 12.35 MJ.kg⁻¹ of metabolizable diet (tab. 1) composed of a pelleted concentrate.

Table 1.

Chemical composition (g.kg ⁻¹) of the experimental diet	
Component	
Dry matter	926.26
Crude protein	192.06
Fat	36.08
Fibre	135.79
Non-nitrogen compounds	483.56
Ash	78.78
Organic matter	847.49
Calcium	9.73
Phosphorus	6.84
Magnesium	2.77
Sodium	1.81
Potassium	10.94
Metabolizable energy	12.35 MJ.kg ⁻¹

Animals were divided into two control groups (C1 and C2) and experimental groups (E1 – E6). Experimental groups received quercetin (Sigma Aldrich, Saint Louis, USA) in injectable form (intramuscularly) at 10 µg.kg⁻¹ in E1 and E2 group, 100 µg.kg⁻¹ in E3 and E4 group and 1000 µg.kg⁻¹ in E5 and E6 group without T-2 toxin for 90 days. T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) to C2, E2, E4 and E6 group at dose 0.08 mg per kg of body weight 72 hours before slaughter intramuscularly was applied. Control groups received injection water (Imuna Pharm a.s. Šarišské Michaľany, Slovak Republic).

In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3.

Table 2.

Application of quercetin and T-2 toxin in rabbit blood *in vivo*

Group	Quercetin ($\mu\text{g.kg}^{-1}$)	T-2 toxin (mg.kg^{-1} of body weight, 72 hours before slaughter)
C1	0	0.00
C2	0	0.08
E1	10	0.00
E2	10	0.08
E3	100	0.00
E4	100	0.08
E5	1000	0.00
E6	1000	0.08

C – control group, E1 – E6 – experimental groups with various doses of quercetin alone or in combination with T-2 toxin

Plate diluting method

Determination of CFU counts: Plate diluting method was applied for quantitative CFU counts determination of respective groups of microorganisms in 1 g of substrate. Gelatinous nutritive substrate in Petri dishes was inoculated with 1 mL of chyme samples pour plate method in three replications. Homogenized samples of faecal chyme (chyme was taken to sterile Petri dishes) were prepared in advance by sequential diluting based on decimal dilution system application. Enterococci were counted on Slanetz-Bartley agar (Biolife, Italy) and incubated at 37 °C for 48-72 hours, lactobacilli were counted on MRS Lactobacillus agar (Biolife, Italy) and incubated at 37 °C for 48-72 hours and coliforms bacteria were counted on MacConkey agar (Biolife, Italy) and incubated at 37 °C for 24-48 hours. Isolated species, genera and groups of microorganisms and their fundamental identification were performed as per standard norms (*Holt et al., 1994*).

Results and discussion

The digestive system of animals hosts several hundred species of bacteria. These bacteria (microbial flora or microflora) in the gastrointestinal tract form a complex ecosystem that plays an important role in maintaining the integrity of the host's enterocyte, providing enzymes for metabolism of ingested of foods, modulation of metabolic processes for host needs (such as converting steroids and unconjugating bilirubin into more water soluble forms of urobilinogen), and protecting the host epithelial cells from colonization by toxin-producing or invasive intestinal pathogens. The intestinal microflora also provide certain nutrients and vitamins that are beneficial to the host. The relationship between the non-harmful microbiota and the host is often viewed as symbiotic; therefore, the organisms in the gastrointestinal tract are commensals under normal conditions. A complete understanding of the balance and composition of the gut microflora in the host animal species remains incomplete (*Fortun-Lamothe and Boullier, 2007*).

While lactobacilli, coliforms and streptococci constitute the main components of the intestinal microflora in the majority of farm animals, in the rabbit these bacteria can be found in traces only. In the first week of life their number is usually between 10^2 and 10^8 , but subsequently they "disappear" and their count falls below 100. In the present experiment, the free nursing method (Group A) resulted in higher lactobacillus counts throughout and also higher coliform counts in the first four days of life. In Group C, a major rise was found in the coliform count by day 6, and on day 10 the coliform count was still higher than in the other two groups. This was presumably due to the slower establishment of the main microflora (i.e. the slower rate of colonisation of the caecum by *Bacteroides*). Although it could be established that the *Lactobacillus* and coliform counts were in inverse ratio to the *Bacteroides*

count, a clear antagonism between the normal bacterial components of the microflora could still not be demonstrated (*Kovacs et al., 2004*).

In our study the number of coliforms bacteria ranged between 4.23 to 5.50 log CFU.g⁻¹. The higher number of coliforms bacteria was found in control group without any application. The lower number of coliforms bacteria was found in experimental group with 1000 µg.kg⁻¹ of querticin without T2 toxin application.

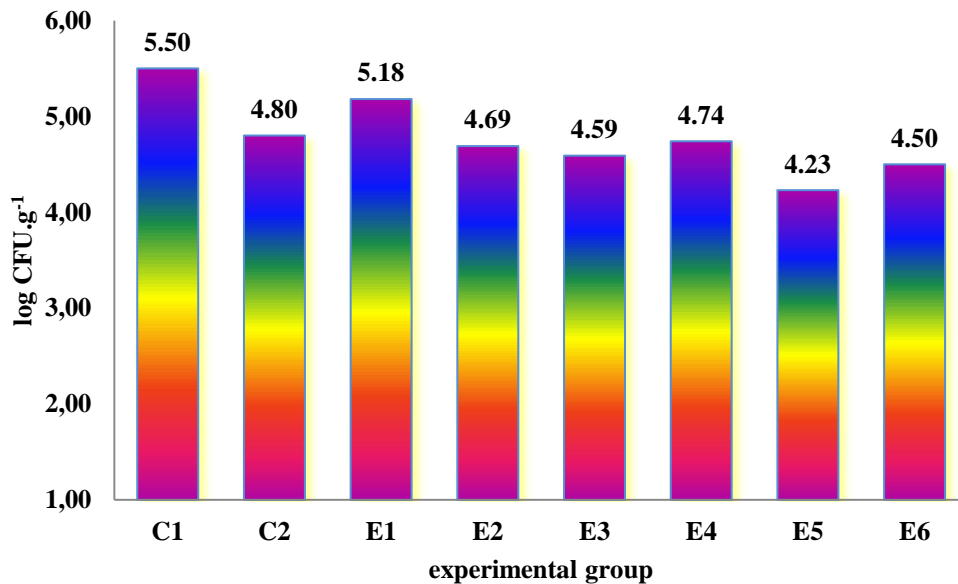


Figure 1:

The number of coliforms bacteria in caecum of rabbits

In our study the number of lactobacilli ranged between 3.20 to 4.54 log CFU.g⁻¹. The higher number of lactobacilli was found in experimental group with 100 application µg.kg⁻¹ of quertecin without T2 toxin application. The lower number of lactobacilli was found in control group without quertecin with 0.08 mg.kg⁻¹ T2 toxin application.

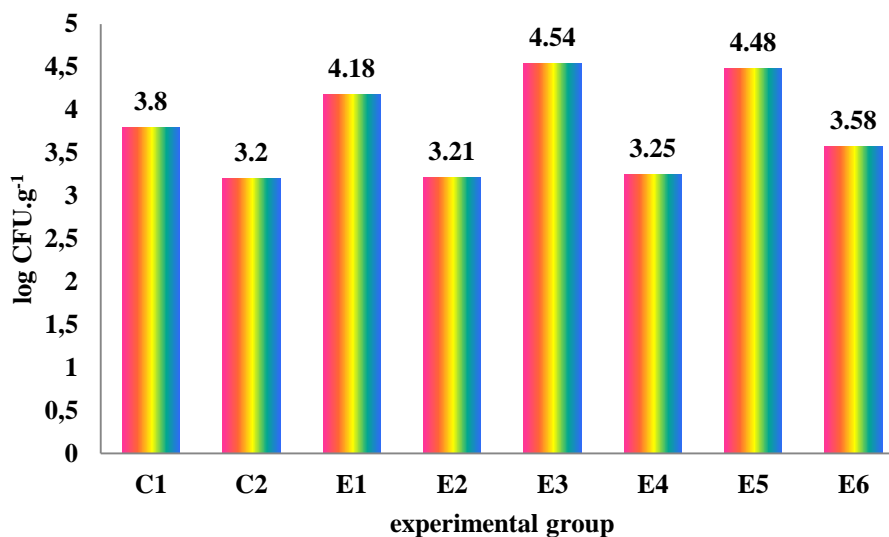


Figure 2:

The number of lactobacilli in caecum of rabbits

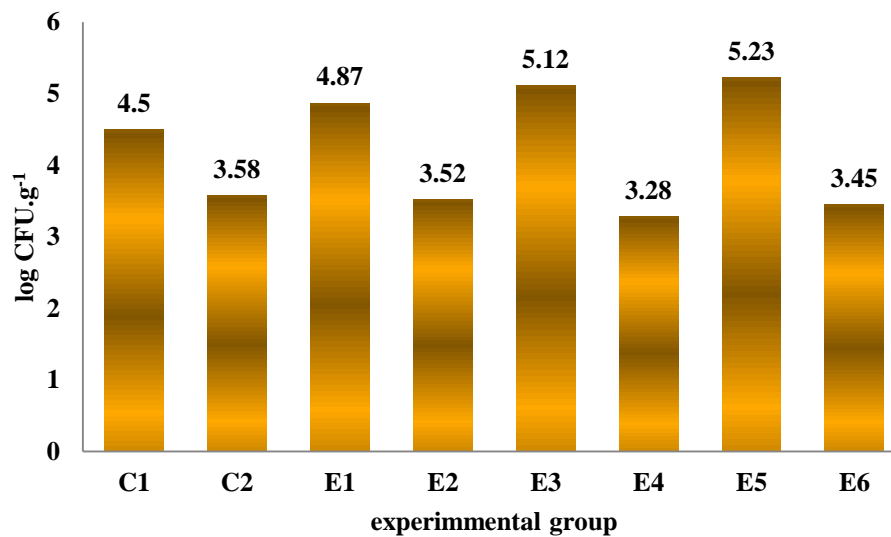


Figure 3:

The number of enterococci in caecum of rabbits

In our study the number of enterococci ranged between 3.28 to 5.23 log CFU.g⁻¹. The higher number of enterococci was found in experimental group with 100 application $\mu\text{g.kg}^{-1}$ of quertecin with 0.08 mg.kg⁻¹ T2 toxin application. The lower number of enterococci was found in control group with 1000 quertecin $\mu\text{g.kg}^{-1}$ without toxin application.

The flora of the rabbit gastrointestinal tract varies with age, diet, and antibiotic use. The suckling rabbit maintains a relatively sterile stomach by the presence of a substrate in the doe's milk and an enzyme in the suckling rabbit's stomach. The predominant microflora in the lower gastrointestinal tract of young rabbits is streptococci and enterobacteria, whereas the predominant inhabitant of the adult rabbit small intestine, cecum, and colon is *Bacteroides*. The low gastric pH (1 to 1.9) of the adult maintains a relatively sterile stomach in this age group as well (Funn, 2001).

The adult rabbit GI tract is inhabited predominantly by the nonsporulated gram-negative bacilli *Bacteroides* (strict anaerobe) in the small intestine, caecum, and colon. A smaller number of sporulated anaerobes (*Endosporus* before weaning and *Acuformis* after weaning) can also be found in tile cecum and colon. Previous studies on *Lactobacillus* rarely identified this organism in any part of the rabbit's GI tract, regardless of age. A recent study, however, found *Lactobacillus confusus* in rabbit fecal samples (Funn, 2001).

Conclusion

The supply of rabbits with qertecin and T2 toxin and their access to the doe's faeces have been shown to affect the development of the caecal microflora.

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