

CHARACTERIZATION OF RABBITS MICROFLORA AFTER NATURAL PRODUCTS APPLICATION

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ABSTRACT

The aim of this study was to compare the microbial species in *caecum* microflora of rabbit in control group against experimental group where epicatechin and patulin were applied. It is first study about *caecum* microflora study after epicatechin and patulin application. In this study classical method was used for enumeration of bacteria. 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. In our study the number of coliforms bacteria ranged between 3.78 to 4.45 log cfu.g⁻¹, lactobacilli ranged between 3.85 to 5.00 log cfu.g⁻¹ and enterococci ranged between 3.48 to 4.48 log cfu.g⁻¹.

Keywords: gastrointestinal microflora, epicatechin, patulin, rabbits

INTRODUCTION

The rabbit has some unique anatomical features including the *sacculus rotundus* and the *vermiform appendix*. Gastrointestinal disorders in these animals can be a challenge to clinicians as not only the motility of the hindgut must be maintained, but the microflora as well. Dysbiosis, or changes in the microflora can release toxins and further alter the pH, microflora and motility. The clinician must also be aware of gastrointestinal pain and hydration status accompanying most gastrointestinal disease (Johnson-Delaney, 2006).

The *caecum* is the largest part of the digestive tract usually containing up to 65% of the gastrointestinal contents. It is large, thin-walled, and fills most of the left ventral abdomen. It measures approximately 15–20 cm in length. It has 3 white muscular longitudinal bands: the dorsal, ventral, and medial *teniae coli*. The *sacculus outpouchings* between the bands are *haustra*. The colon appears dark green and is approximately 70 cm long. It functionally is divided into the shorter proximal section (20 cm) and the distal, longer section (50 cm). The proximal colon has mucosal folds on the mesenteric side that forms a longitudinal furrow. The furrow aids in separating high protein and smaller particles from the poorer quality material that will pass out of the colon as dry fecal pellets. Antiperistalsis transports the bacteria and higher protein particles back to the *caecum* for further fermentation (O'Malley, 2005).

Antibiotic administration has been linked to disruption of normal gut flora. A generality is that broad-spectrum antibiotics administered subcutaneously or intramuscularly are less likely to cause problems (Flecknell, 2002). Probiotics along with other functional foods positively affect the health of the consumer (Horská, 2012). The metabolism of flavonoids has not yet been well characterized. Absorbed flavonoids are present in the common blood circulation in the form of glucuronide, sulfate and methylate conjugates and are excreted via urine or bile. In addition, several metabolites of ingested flavonoids are formed by the intestinal microflora. Microorganisms are responsible for hydrolysis of flavonoid conjugates as well as for the formation of easily absorbed flavonoid ring fission products. A number of new reports on flavonoid absorption have been published recently. Despite on the fact that a number of *in vivo* studies have been carried out in rats, pharmacokinetics behavior of epicatechin has not been fully understood. Compare with rats, rabbits physiology is fairly similar to that of

humans, and rabbits were used as an animal model for pharmacokinetic study (Chen and Hsu, 2009).

Patulin is a toxic chemical contaminant produced by several species of mold, especially within *Aspergillus*, *Penicillium* and *Byssoschlamys*. Exposure to this mycotoxin is associated with immunological, neurological and gastrointestinal outcomes. A full understanding of the molecular genetics of patulin biosynthesis is incomplete, unlike other regulated mycotoxins (aflatoxins, trichothecenes and fumonisins), although the chemical structures of patulin precursors are now known (Puel *et al.*, 2010).

The purpose of this study was to compare the microbial species in gastrointestinal microflora of rabbits in control group against experimental group where epicatechin and patulin were applied. It is first study about *caecum* microflora study after epicatechin and patulin application. In this study for enumeration of bacteria were using classical method.

The aim of this study was to compare the microbial species in *caecum* microflora of *rabbit* in control group against experimental group where epicatechin and patulin were applied.

MATERIAL AND METHODS

Animals and diet

Adult female rabbits (n = 16), 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 (Table 1) composed of a pelleted concentrate. Animals were divided into four groups: control group (C) without addition of natural products, experimental group (E1) with addition of epicatechin (10 µg.kg⁻¹), experimental group (E2) with addition of patulin (10 µg.kg⁻¹) and experimental group (E3) with combined addition of epicatechin (10 µg.kg⁻¹) and patulin (10 µg.kg⁻¹). Animals from experimental group received

patulin through intramuscular injection twice a week for two weeks. 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 ethical committee.

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

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

In commercial rabbit meat production, a major part of the mortality results from diseases of the digestive system that are related mainly (about 25%) around weaning (between days 18 to 50). Beside economic losses, this disease has a negative influence on animal welfare as well. Antibiotics are still widely used to reduce mortality of the growing rabbit, although there is an increasing human health and food safety concern over drug residues in meat products (Bónai et al., 2008).

The caecal microflora and the fermentation processes taking place in the caecum play a key role in the digestion of rabbits. Imbalance of the intestinal microflora (dysbiosis) plays a direct or indirect role in the development of digestive disturbances or diseases (Kovacs et al., 2003).

In our study the number of coliforms bacteria ranged between 3.78 in experimental group with patulin to 4.45 log cfu.g⁻¹ in experimental group with patulin and epicatechin. 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 10 µg.kg⁻¹ of patulin without epicatechin application (fig. 1).

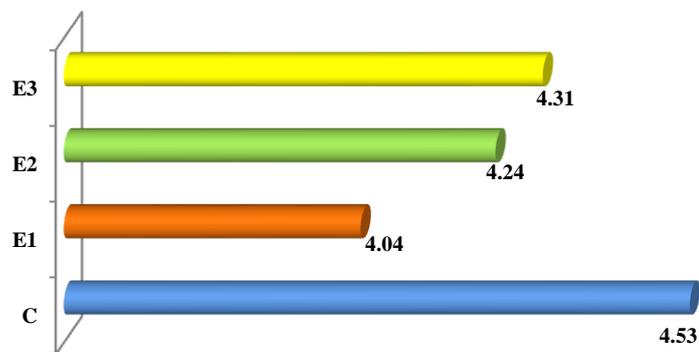


Figure 1 The average number of coliforms bacteria in caecum of rabbits in log cfu.g⁻¹, C - without addition of natural products, E1 - experimental group with addition of epicatechin (10 µg.kg⁻¹), E2 - experimental group with addition of

patulin (10 µg.kg⁻¹), E3 - experimental group with combined addition of epicatechin (10 µg.kg⁻¹) and patulin (10 µg.kg⁻¹)

In our study the number of lactobacilli ranged between 3.85 in experimental group with patulin and epicatechin to 5.00 log cfu.g⁻¹ in control group The higher number of coliforms bacteria was found in control group without any application. The higher number of lactobacilli was found in experimental group with 10 µg.kg⁻¹ of patulin without epicatechin application (fig. 2).

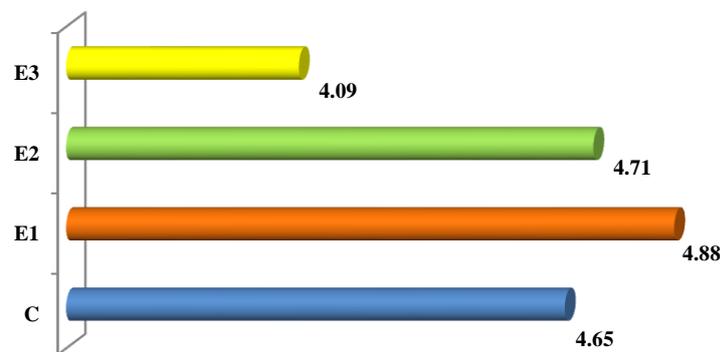


Figure 2 The average number of lactobacilli in caecum of rabbits in log cfu.g⁻¹ C - without addition of natural products, E1 - experimental group with addition of epicatechin (10 µg.kg⁻¹), E2 - experimental group with addition of patulin (10 µg.kg⁻¹), E3 - experimental group with combined addition of epicatechin (10 µg.kg⁻¹) and patulin (10 µg.kg⁻¹)

In our study the number of enterococci ranged between 3.48 in experimental group with patulin to 4.48 log cfu.g⁻¹ in control group The higher number of coliforms bacteria was found in control group without any application. The higher number of enterococci was found in experimental group with 10 µg.kg⁻¹ of patulin with epicatechin application (fig. 3).

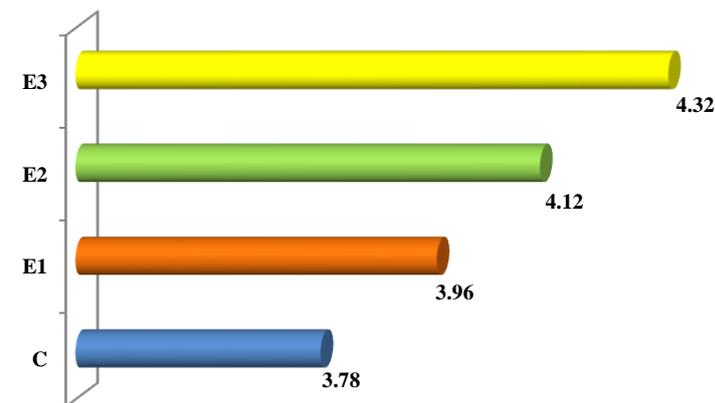


Figure 3 The average number of enterococci in caecum of rabbits in log cfu.g⁻¹ C - without addition of natural products, E1 - experimental group with addition of epicatechin (10 µg.kg⁻¹), E2 - experimental group with addition of patulin (10 µg.kg⁻¹), E3 - experimental group with combined addition of epicatechin (10 µg.kg⁻¹) and patulin (10 µg.kg⁻¹)

The aim of Kačaniová et al., 2013 study was to compare the microbial species in gut microflora of rabbit in control group against experimental group were it was applied qertecin and T2 toxin. In this study, classical methods were used for enumeration of bacteria. 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. In this study the number of coliforms 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, similarly at in our study with epicatechin and patulin.

CONCLUSION

One of the most interesting aspects of a rabbit's body is his digestive system. Unlike a others animals, rabbits can eat a wide variety of plant material. They can process and extract nutrients from many plants that are indigestible to less adaptable herbivores or omnivores. This ability helps make rabbits highly successful in a variety of environments around the world. Understanding how

your rabbit's digestive system functions is important so that you can feed him in a way that's most efficient for his body. The supply of rabbits by epicatechin and patulin and their access to the doe's faeces have been shown to affect the development of the caecal microflora.

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