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Effect of rabbit-origin enterocin-producing probiotic strain *Enterococcus faecium* CCM7420 application on growth performance and gut morphometry in rabbits

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ABSTRACT: The interactions between probiotic bacteria and the immune system in animals are known, but particularly in rabbits have not been sufficiently documented. Therefore the present study deals with the effect of the enterocin-producing probiotic strain *Enterococcus faecium* CCM7420 on the growth performance and jejunal morphometry in rabbits. Forty-eight weaned rabbits of male sex (NZB breed) at 5 weeks of age were randomly divided into experimental (EF) and control group (CG) with 24 rabbits in each group. Each day of the treatment period (21 days), the rabbits in the EF group were administered a fresh culture of the *E. faecium* CCM7420 strain (5.0×10^8 CFU/animal/day in their drinking water); for the next 21 days after probiotic cessation the rabbits had untreated drinking water (the whole experiment lasted for 42 days). All animals had access to water *ad libitum*. Three animals from each group were randomly selected for slaughter at days 21 and 42 of the experiment to measure morphological parameters (villus height (VH), crypt depth (CD), VH : CD ratio) of the intestinal tissue in the proximal jejunum. The average daily gain was higher in EF group (39.49 g) than in CG (39.04 g). The jejunum VH, surface area, and VH : CD ratio increased, while CD decreased in EF group compared to CG. Inferring from these results, we conclude that administration of *E. faecium* CCM7420 strain to rabbits may improve weight gain due to better utilization of feed and larger absorption surface in the gut and also may positively influence the health status via enhancing the gut health in rabbits.

Keywords: probiotic; growth parameter; functionality; food-producing animals

INTRODUCTION

Weaning is a stressful period related to large economic losses in rabbits husbandries. It can increase the susceptibility of animals to several infections. The ban on antibiotic growth promoters in animal diets by the European Union has led to exploratory research into their alternatives on a natural basis. The use of probiotics, prebiotics, bacteriocins, herbal extracts or organic acids showed promising results in enteric disease prevention, enhancement of growth performance, carcass quality, and immune response in rabbits (Matusevicius et al. 2004; Falcao-e-Cunha et al. 2007; Kritas et al. 2008; Pogany Simonova et al. 2009; Oso et al. 2013). It is well known that probiotic supplementation improves growth rate, enhances efficiency of feed conversion in rabbits, and also influences the intestinal microbiota through the action of beneficial microbes (Pogany Simonova et al. 2009). The stable or improved intestinal environment and gut health directly influence the health status and growth performance of animals due to better nutrient absorption in the gut. Although there are several scientific reports about the interaction between natural additives and the immune system in rabbits (Cardinali et al. 2008; Szaboova et al. 2012; Pogany Simonova

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et al. 2013), their effect on the rabbit intestinal morphology and intestinal barrier function has not been documented sufficiently (Oso et al. 2013). For this reason, the effects of bacteriocinogenic probiotic strain *Enterococcus faecium* CCM7420 on the growth performance and the jejunal morphology in rabbits were studied.

MATERIAL AND METHODS

Forty-eight weaned rabbits of male sex (NZB breed) were randomly divided into experimental (EF) and control group (CG) with 24 rabbits in each at 5 weeks of age. The rabbits were housed in standard cages (0.61 m × 0.34 m × 0.33 m; type D-KV-72 supplied by Kovobel, Domažlice, Czech Republic), two animals per cage. A 16 h light/8 h darkness cycle was kept throughout the experiment. Temperature and humidity were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed the air temperature in the building to be maintained within 16 ± 4°C during the experiment. Relative humidity was about 70 ± 5%. Daily, at the same time in the morning, the rabbits in the EF group had a fresh culture of *E. faecium* CCM7420 strain (5.0×10^8 CFU/animal/day) administered into their drinking water. *E. faecium* CCM7420 strain doses were administered to the rabbits for 21 days (treatment period). The CCM7420 strain is an enterocin-producing probiotic isolate from rabbit faeces, isolated and tested in our laboratory (Simonova 2006; Pogany Simonova et al. 2009). The experiment lasted for 42 days (first 21 days for CCM7420 probiotic administration and the following 21 days after probiotic cessation). The rabbits were fed a granulated feed (complete granulated mixture; pellets have 3 mm in diameter) *ad libitum*. The chemical composition of the diet was as follows: 197.0 g/kg of crude protein, 166.5 g/kg of crude fibre, 970.0 g/kg of dry mater, 34 g/kg of fat, 139 g/kg of starch, 80.0 g/kg of ash, 10.0 MJ/kg of metabolizable energy.

Body weight was checked daily; average daily weight gain and feed conversion were calculated mathematically. Mortality and morbidity were also recorded in groups daily.

Three animals from each group were randomly selected for slaughter at days 21 and 42 of the experiment; they were stunned with electronarcosis (90 V for 5 s), immediately hung by the hind legs on the processing line and quickly bled by cutting the jugular veins and the carotid arteries. To test morphometry (villus height (VH), crypt depth (CD), and VH : CD ratio), intestinal tissue (1 cm^2) from the proximal jejunum was sampled and treated as previously described by Zitnan et al. (1998). All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals (1996) and the trials were accepted by the Ethical Commission at the Institute of Animal Physiology in Košice and by the Slovak Veterinary and Food Administration. Statistical evaluation of the results was performed using a non-paired Student's *t*-test with the level of significance set at $P < 0.05 \pm SD$.

RESULTS AND DISCUSSION

All animals were in a good health condition throughout the trial with no symptoms of disorders. The average daily gain was higher in the EF (39.49 g) than in the CG group (39.04 g). Improved feed conversion was also noted in the EF group (EF: 2.63 vs CG: 2.91 between days 35–56 of age = 21 days of the CCM7420 strain administration (g/g); EF: 4.01 vs CG: 4.09 between days 56–77 of age = 21 days after the CCM7420 cessation (g/g); EF: 3.11 vs CG: 3.43 between days 35–77 of age = throughout the whole experiment). Body weight gain is the most frequently checked parameter during additive application. The application of probiotics is associated with their beneficial effect on body weight, morbidity and/or mortality in rabbit farms (Matusevicius et al. 2004; Kritas et al. 2008); improved weight gain and feed conversion ratio are in agreement with our previous findings (Pogany Simonova et al. 2009). Oso et al. (2013) described the poor effect of probiotics on rabbits growth rate. The improvement in growth and feed efficiency could be explained by the retention of beneficial microbiota in the gut and improving feed digestion and absorption due to improved intestinal morphology. Another explanation could be that the probiotics can act on nondigestible carbohydrates and give rise to short-chain fatty acids, which can affect a better absorption of minerals and nutrients. We also hypothesize on the enlargement of the absorption surface due to enterocyte proliferation; improved morphometry parameters, e.g. increased VH and

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Table 1. Effect of *Enterococcus faecium* CCM7420 (EF) administration on the growth performance and jejunal morphometry in rabbits

	EF	CG	*P*-value
Growth performance			
Number of rabbits	(*n* = 24)	(*n* = 24)	
Initial live weight (35 days) (g)	966.25 ± 143.52	985.00 ± 105.82	0.6089
Intermediate live weight (56 days) (g)	1 890.00 ± 139.61	1 892.08 ± 91.93	0.9517
Final weight (77 days) (g)	2 625.00 ± 182.03	2 624.76 ± 68.24	0.9952
Morphometry at 56 days			
Villus cut surface (μm^2)	81 150.0 ± 2 527.0	80 610.0 ± 3 055.0	0.8260
Villus height (μm)	68.02 ± 18.7	668.0 ± 29.0	0.5302
Crypt depth (μm)	178.7 ± 11.6	180.7 ± 16.6	0.8723
Villus height : crypt depth	3.83 ± 0.35	3.73 ± 0.49	0.7749
Morphometry at 77 days			
Villus cut surface (μm^2)	81 290.0 ± 1 176.0	80 830.0 ± 2 436.0	0.7803
Villus height (μm)	677.0 ± 10.8	673.0 ± 20.5	0.7800
Crypt depth (μm)	180.0 ± 7.02	179.0 ± 11.6	0.9681
Villus height : crypt depth	3.77 ± 0.21	3.77 ± 0.36	0.9896

EF = experimental group administered a fresh culture of *E. faecium* CCM7420 strain (5.0 × 10^8 CFU/animal/day), CG = control group

decreased CD, were measured (Table 1). These results suggest an increased surface area capable of greater absorption of available nutrients. Throughout the *E. faecium* CCM7420 strain administration the jejunum to CG surface area and VH : CD ratio also increased; these results could support the hypotesis about the enlargement of the absorption surface. The effect of dietary treatments on intestinal morphology has been already described/reviewed mainly in chickens, pigs, and rats, but data on the rabbit gut morphology are rare (Matur and Eraslan 2012). Oso et al. (2013) described improved morphological parameters in the rabbit ileum (increased villi length) after prebiotics inclusion compared with the probiotic group. In chickens, the highest VH and VH : CD were obtained in the jejunum by Primalac® (*Lactobacillus*/*Streptococcus* cultures) supplementation (Yakhkeshi et al. 2011), similarly to our findings.

The administration of *E. faecium* CCM7420 strain to the rabbits altered their growth performance and their jejunum morphometry as well. These changes may influence the gut function and health and may improve the nutrient uptake.

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