Effect of beneficial strain *Enterococcus faecium* EF9a isolated from Pannon White rabbit on growth performance and meat quality of rabbits

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**ABSTRACT**

Rabbit meat is highly appreciated for its high nutritional and dietetic quality, which could be enhanced by natural additives. Therefore, in this study, the effect of bacteriocinogenic and probiotic strain *Enterococcus faecium* EF9a isolated from Pannon White rabbits was checked on growth and meat quality (pH, colour, water holding capacity (WHC), protein, fat and energy content) of broiler rabbits in a model experiment. The average daily weight gain was higher in EF9a group (*p* < .0001). The treatment did not have a negative influence on the pH, colour, WHC, protein and fat contents or energy value of the rabbit carcase. Inferring from these results we conclude that the application of this new bacteriocinogenic *E. faecium* EF9a strain with probiotic properties could be promising in rabbit farms.

**HIGHLIGHTS**

- The new bacteriocinogenic *E. faecium* EF9a strain could be used in rabbit farms.
- The EF9a strains application increased average daily weight gain.
- Treatment with the EF9a strain does not affect meat quality.

**Introduction**

The demand for healthy meat and meat products in consumers is steadily increasing. Therefore, researchers and farmers are trying to find the most appropriate alternatives to obtain healthy and high valued meat and meat products. Rabbit meat is appreciated due to its high nutritional and dietetic properties: its high content of proteins, polyunsaturated fatty acids (PUFA), potassium, phosphorus, magnesium and low amounts of fat, cholesterol and sodium. Its energy value is comparable to various commonly used and consumed sorts of red meat (Dalle Zotte 2002). For these reasons, the rabbit meat is better digested and recommended mainly for children and persons with cardiovascular illnesses.

Previously, there have been many alternatives, such as probiotics, prebiotics, organic acids, herbs and herbal extracts, enzymes, proteins – e.g. enterocins, fatty acids, vitamins and selenium have been tested in rabbits to stabilise and improve health, productivity and economy of rabbit farms. There are many papers dealing with the rabbit meat composition, its physicochemical, nutritional, technological properties and with effects of natural additives on rabbit carcase quality (Dalle Zotte 2002; Dalle Zotte and Szendro 2011; Pogány Simonová et al. 2010, 2016; Dal Bosco et al. 2014; Matics et al. 2017). Other articles have presented results concerning probiotics application on rabbits health, growth, immunity, digestion, gastrointestinal microflora and enzymatic activity (Matusevičius et al. 2006; Kritas et al. 2008; Simonová et al. 2008; Pogány Simonová, Lauková et al. 2009; Simonová et al. 2013; Kalma et al. 2016; Bhatt et al. 2017; Lauková et al. 2019). Although the administration of probiotics on rabbit meat quality have been investigated in several papers (Matusevičius et al. 2006; Pogány Simonová, Szabóová et al. 2009; Pogány Simonová et al. 2016), further investigation is needed to develop the already known facts and to achieve new and more results concerning the physicochemical properties.

*Enterococcus faecium* EF9a is a bacteriocin-producing strain with probiotic properties isolated.
from the faeces of Hungarian rabbit breed Pannon White in the Laboratory of Animal Microbiology (IAP CBs SAS) and tested under in vitro and in vivo conditions by our team (Lauková et al. 2019). Because of our previous application of beneficial E. faecium strains (Pogány Simonová, Lauková et al. 2009; Pogány Simonová, Szabóvá et al. 2009; Lauková et al. 2012) using different breeds Hycole, Hyplus (Pogány Simonová, Lauková et al. 2009; Pogány Simonová, Szabóvá et al. 2009; Simonová et al. 2010; Lauková et al. 2012; Simonová et al. 2013; Pogány Simonová et al. 2016) spreading the ‘target of our beneficial isolates’ and we decided to determine the EF9a strain dietary effect on growth performance and some carcass characteristics.

Materials and methods

Experiment schedule, diet, and sampling

Fourty-eight rabbits of both sex (Hyplus breed) aged five weeks were weaned and randomly divided into experimental (EG, average weight of rabbits was 1001.0 ± 114.0 g) and control groups (CG, average weight of rabbits was 1001.0 ± 114.0 g) with 24 rabbits in each. The rabbits were housed in standard cages (0.61 m × 0.34 m × 0.33 m, of the type D-KV-72 supplied by the Kovobel company, Domažlice, Czech Republic), two animals per cage. A cycle of 16 h of light and 8 h of dark was used 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 air temperature in the building to be maintained within 16 ± 4 °C during the experiment. Relative humidity was about 70 ± 5%. The cages allowed faeces separation. The animals were fed commercial pellet diet for growing rabbits (Tekro Nitra Ltd, Párovské Háje-Vráble, Slovakia; Table 1) during the experiment with access to water ad libitum. The metabolisable energy (ME) content was calculated using the equation of Wiseman et al. (1992). Chemical analyses were conducted according to Association of Official Analytical Chemists (1995), with the considerations given by Gidenne et al. (2001) for dry matter, crude protein, crude fibre, crude fat, ash and organic matter. During four weeks, every day at the same time in the morning, the rabbits in the EG were administered cells of E. faecium EF9a strain in Ringer solution (1.0 × 109 CFU/mL, in a dose 500 μL/animal/day) into water, prepared according to Pogány Simonová, Lauková et al. (2009); these four weeks were the treatment. On the base of our previous experiments, we had information about the volume of water drunk by rabbits; the additives were applied firstly to 100 mL of drinking water in all cages and after consuming this volume the rabbits had access to water ad libitum. Control rabbits (group C) had the same conditions, but without EF9a application into drinking water. The presence and counts of the EF9a strain was monitored and controlled during the whole experiment in mixture faecal samples (days 28 and 42; five samples/group/day) and individually in caecal samples (days 28 and 42; six samples/group/day) and analysed by standard microbiological method on Todd-Hewitt agar (Imuna) enriched with rifampicin (100 μg/mL; counts of EF9a strain in faeces – day 28: 3.99 CFU/g; day 42: 0.90 CFU/g; counts of EF9a strain in caecum – day 28: 0.90 CFU/g; day 42: 1.96 CFU/mg). From day 28 (63 day of age) all animals were fed only the commercial diet. The model experiment lasted for 42 days (77 day of age). Weight feed mixture was checked daily and average daily weight gain (ADWG) and feed conversion were calculated mathematically, as well as mortality, at the end of the experiment.

Table 1. Ingredients and determined chemical composition of the commercial diet.

| Chemical analysis, minerals and vitamins | Feed ingredients (%) | Diet |
|----------------------------------------|----------------------|------|
| Dry mattera                              | 885.7                | Dehydrated lucerne meal 41.4 |
| Crude proteina                           | 181.3                | Extracted sunflower meal 7.8 |
| Crude fibrea                             | 173.8                | Oats 12.8 |
| Fatb                                    | 36.1                 | Wheat bran 32.9 |
| Ashb                                    | 71.3                 | Dry malting sprouts 2.0 |
| Organic compoundsb                       | 814.4                | Limestone 0.9 |
| Starcha                                 | 139.4                | PXM-KV C 1% (Tekro) 1.4 |
| Calciuma                                | 9.9                  | Soybean oil 0.5 |
| Phosphorusa                             | 3.4                  | Sodium chloride 0.3 |
| Magnesiuma                              | 2.1                  | | |
| Sodiuma                                 | 1.8                  | | |
| Potassiuma                              | 11.8                 | | |
| Irona                                   | 530.5                | | |
| Zinca                                   | 190.6                | | |

aExpressed in g/kg feed.

bThe ash and organic compounds are expressed based on dry matter content.

Expressed in mg/kg feed.

The PXM-KV C is mixture of vitamins.
Six animals from each group were slaughtered at days 28 and 42 of the experiment group and samples were taken. After electro-stunning (50 Hz, 0.3 A/rabbit/4 s), rabbits were slaughtered in an experimental slaughterhouse by cutting the carotid and jugular veins and bleeding out. Musculus longissimus dorsi (MLD) was separated by removing the skin and connective tissue chilled and stored at 4°C for 24 h until physicochemical analysis started.

The ultimate pH48 was determined after 48 h (post-mortem) using a Radelkis OP-109 (Jenway, England) with a combined electrode penetrating 3 mm into samples. Colour characteristic were expressed by CIE L*a*b system (lightness-L*, 0: black and 100: white), redness and greenness-a*; yellowness and blueness-b*) at 24 h after bleeding using a Lab Miniscan (HunterLab, Reston, VA). Lightness measurements at room temperature were also taken. The content of water, protein and fat were estimated using an INFRAtec 1265 (CM Instruments Laboratörgerate GmbH, Bünde, Germany) spectroscope and expressed in g/100 g; from these values, the energy value was calculated by the equation of Strmiska et al. (1988): EV (kJ/100 g) = 16.75 x protein content (g/100 g) + 37.65 x fat content (g/100 g). The ash content was determined by mineralisation of samples at 550°C according to STN 570185. Water holding capacity (WHC) was determined by compress method at constant pressure (Hasek and Palanska 1976).

Statistical evaluation of the results was performed using Student t-test. The results are quoted as means ± SD.

Results and discussion

All animals were in good health condition throughout the trial during the E. faecium EF9a application; no morbidity and mortality was noted. There was a higher live weight at 63 days of age (+34 g; p < .0001), final live weight at 77 days of age (+158 g; p = 0.0483) and ADWG between 64 and 77 days of age (+8 g/d; p < .0001) in the rabbits of EG group (Table 2). In this respect, also another authors observed the beneficial effects of feed supplementation with probiotics on body weight of rabbits (Matuszecvics et al. 2006; Kritas et al. 2008; Kalma et al. 2016). Furthermore, improved ADWG was also noted during previous experiments with both autochthonous and non-autochthonous probiotic strains of E. faecium administration in rabbits (Pogány Simonová, Lauková et al. 2009; Lauková et al. 2016), which agrees to some extent with the results of the present study. Surprisingly, in the present study, during EF9a administration ADWG increased only slightly (2.1%) compared to rabbits of the control group, while after the cessation of probiotic administration significantly higher ADWG was noted (p < .0001; by 20.8%). We suppose that EF9a application had prolonged effect on the rabbit’s growth, which was noted after cessation, in contrast to the CCM7420 strain, when higher ADWG was already noted during the treatment period (Pogány Simonová, Lauková et al. 2009). The improved weight gains could be explained in terms of more intensive metabolism in the caecum; our hypothesis could be confirmed also by better VFA production during the treatment.

No statistically significant difference appeared between CG and EG groups of rabbits regarding the meat quality – pH24 (24 h post mortem), colour and chemical composition (Table 3). Most reports present higher pH24 values than 5.70 (Dalle Zotte 2002; Polak et al. 2006). In our study, lower pH48 (48 h post mortem, comparing to pH24, which is usually tested) value (5.65) was obtained, which could be explained by depletion of the glycogen reserve in muscles during

| Table 2. The effect of E. faecium EF9a administration (means ± SEM) on the growth performance and carcass weight of rabbits. |
|---------------------------------------------------------------|
|                | EG                  | CG                  | p Value |
| Initial live weight (day 1), g (n = 24/group)               | 1001.0 ± 134.0      | 1007.0 ± 105.0      | .9791   |
| Intermediate live weight (day 28), g (n = 24/group)         | 2059.0 ± 192.0*     | 2025.0 ± 212.0**    | <.0001  |
| Final weight (day 42), g (n = 18/group)                      | 2574.0 ± 230.0      | 2416.0 ± 178.0      | .0483   |
| Average daily weight gain between days 1–28 (g/d)           | 35.00 ± 5.24        | 34.25 ± 9.32        | .8907   |
| Average daily weight gain between days 28–42 (g/d)          | 38.50 ± 1.50        | 30.50 ± 2.50*       | <.0001  |
| Average daily weight gain between days 1–42 (g/d)           | 36.16 ± 4.67        | 33.00 ± 7.69        | .3012   |

EG: experimental group of rabbits; CG: control group of rabbits.

*The intermediate (n = 24/group) and final (n = 18/group) live weights were checked at day 28 of the experiment - at the end of the EF9a strain application and day 42 of the experiment- at the end of the EF9a strain cessation before the animal selection and slaughtering.

**Days 1–28: application of E. faecium EF9a into water (EG) for 28 days.

**Days 1–42: time schedule of the whole experiment (application of EF9a strain for 28 days and after the E. faecium EF9a strain cessation between days 28–42).

**Different lowercase letters indicate differences between treatments: p < .001.
reduction in the EF9a group. Fathi et al. surprisingly, after the strain cessation, the ash content effect on ash content during the its application, but The probiotic EF9a supplementation had no significant Lactobacillus acidophilus meat after also, the lipid concentration was higher than in rabbit contradictions between lipids (slightly increase of positive correlation between lipids (slightly increase of fat content) and energy (higher energy content) dur-

Table 3. Biochemical and biophysical composition of M. longissimus dorsi of rabbits (means ± SEM) administrating E.faecium EF9a strain.

|                      | EG (n = 6 at each age) | CG (n = 6 at each age) | p Value |
|----------------------|------------------------|------------------------|---------|
| Day 28 of the experiment (after 28 days EF9a administration) |                      |                        |         |
| pH48                 | 5.65 ± 0.081           | 5.58 ± 0.05            | .5254   |
| \(L^*\) (lightness) | 55.42 ± 0.81           | 52.97 ± 2.71           | .0589   |
| \(a^*\) (redness)   | 2.24 ± 0.87            | 0.65 ± 0.31            | .0726   |
| \(b^*\) (yellowness)| 8.91 ± 1.05            | 8.31 ± 0.92            | .8204   |
| Water content (g/100g) | 76.00 ± 0.36       | 75.53 ± 0.65           | .2539   |
| Protein content (g/100g) | 21.43 ± 0.06     | 22.07 ± 0.49           | .1328   |
| Fat content (g/100g) | 1.57 ± 0.40           | 1.40 ± 0.26            | .5321   |
| Energy value (KJ/100g) | 418.04 ± 14.48     | 422.37 ± 15.40         | .9302   |
| Water holding capacity (g/100g) | 35.62 ± 4.65 | 33.73 ± 2.01           | .3073   |
| Ash (g/100g)         | 1.000 ± 0.050         | 1.000 ± 0.050          | 1.0000  |
| Day 42 of the experiment (after 14 days EF9a withdrawal) |                      |                        |         |
| pH48                 | 5.60 ± 0.18           | 5.57 ± 0.14            | .8792   |
| \(L^*\) (lightness) | 46.91 ± 4.18           | 51.83 ± 0.34           | .1289   |
| \(a^*\) (redness)   | 0.76 ± 0.61            | 0.63 ± 0.43            | .9502   |
| \(b^*\) (yellowness)| 6.29 ± 0.70            | 8.10 ± 1.03            | .1235   |
| Water content (g/100g) | 75.50 ± 0.29       | 75.50 ± 0.53           | .8326   |
| Protein content (g/100g) | 22.33 ± 0.26     | 21.97 ± 0.42           | .4815   |
| Fat content (g/100g) | 1.50 ± 0.17            | 1.53 ± 0.46            | .3785   |
| Energy value (KJ/100g) | 430.04 ± 7.36     | 425.71 ± 6.86          | .0649   |
| Water holding capacity (g/100g) | 31.48 ± 5.10 | 30.47 ± 6.66           | .9572   |
| Ash (g/100g)         | 0.670 ± 0.050          | 1.000 ± 0.050          | .003    |

Different lowercase letters indicate differences between treatments: p < .001.

EG: experimental group of rabbits; CG: control group of rabbits.

Conclusions
This study contributes to updating the other our data in the literature concerning the effect of bacteriocin-producing strains with probiotic properties; in this case E. faecium EF9a application in broiler rabbits on their growth performance and carcase quality. After administration of beneficial strain E. faecium EF9a, higher average daily gain was recorded after 28 days addition of the EF9a strain (p < .0001) in rabbits. EF9a application did not have any negative effect on tested meat parameters. Nevertheless, further investigations are needed to assess the efficacy of E. faecium EF9a in rabbit husbandries, to expand the knowledge of the other meat components, e.g. minerals and amino acids content.

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Ethics approval and consent to participate

All care and experimental procedures involving animals followed the guidelines stated in the Institute of Laboratory Animal Resources ‘Guide for the Care and Use of Laboratory Animals’ (1996). The trials were accepted by the Ethical Commission of Institute of Animal Physiology in Košice and by Slovak Veterinary and Food Administration, within the national VEGA project 2/0006/17.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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