Enterocin 7420 and Sage in Rabbit Diet and Their Effect on Meat Mineral Content and Physico-Chemical Properties

Monika Pogáň Simonová 1, * , L’ubica Chrastinová 2 and Andrea Lauková 1

1 Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, Šoltésovej 4-6, 04001 Košice, Slovakia; laukova@saske.sk
2 Institute for Nutrition, National Agricultural and Food Centre, Hlohovecká 2, 95141 Lužianky, Slovakia; lubica.chrastinova@nppc.sk

* Correspondence: simonova@saske.sk

Abstract: Rabbit meat has outstanding nutritional characteristics—it is a lean meat with low fat, cholesterol and sodium content, with high-biological-value proteins, potassium, phosphorus, selenium, iron and vitamin B12 level. The dietary inclusion of natural bioactive compounds can improve the quality of rabbit meat. The present study evaluated the effect of enterocin 7420 (Ent 7420) and sage (Salvia officinalis) extract on the quality and mineral content of rabbit meat. A total of 96 Hyla rabbits (aged 35 days) were divided into E (Ent 7420; 50 µL/animal/d), S (sage extract; 10 µL/animal/d), E + S (Ent 7420 and sage in combination) and control (C) groups. Additives were administrated in drinking water for 21 days. A significant increase in meat iron (p < 0.01) content was noted; phosphorus and zinc levels were also elevated in experimental groups, compared with control data. Ent 7420 and sage treatment reduced the calcium and manganese (p < 0.01) contents. The physico-chemical traits of rabbit meat were not negatively influenced by treatment. Based on these results, diet supplementation, mostly with Ent 7420 but also in combination with sage, could enhance the quality of rabbit meat mineral, with a focus on its iron, phosphorus and zinc contents.

Keywords: enterocin; sage extract; feed additives; mineral profile; rabbit meat

1. Introduction

Rabbit meat is an excellent source of minerals and trace elements, such as potassium, calcium, phosphorus, and selenium and has the highest concentration of iron in any type of meat. It is rich in vitamins, mainly vitamin B3, B6, B12 and E, and in Omega-3 and six fatty acids. Another advantage of rabbit meat is its low sodium level. For this reason, it is recommended for children, pregnant women and people with high blood pressure. It also contains easily digestible proteins, with low amounts of cholesterol and fat. Even though rabbit meat naturally offers a remarkable nutritious quality, the dietary fortification of rabbits with bioactive compounds has been an increasing trend in recent years, and rabbit meat is becoming a functional food with its superior nutritional properties [1]. Amongst natural feed additives, bacteriocins come to the forefront, not only as commonly used starter cultures and preservatives in food industry, but also in the agriculture sector and veterinary medicine to improve the animals’ health and productivity [2–4]. Bacteriocins, antimicrobial substances produced mostly by lactic acid bacteria (LAB; [5]), are usually used in animals to enhance their health status and productivity, due to stabilized intestinal microbiota and mucosal immunity. Mostly, colicins, microcins, lacticin, garvicin and lantibiotic nisin are used in aquacultures, ruminants, poultry and swine production [3, 6, 7]. Enterocins (bacteriocins produced mostly by enterococci) also have a great antimicrobial and immunostimulatory potential but until recently, mostly enterocin-producing strains were applied to piglets and poultry [8–10]. Only enterocin (Ent) A (produced by the Enterococcus faecium EK13/CCM7419 strain) was in vivo tested in Japanese quails [10]. Rabbits are also a significant part of animal food production, and mostly probiotics and herbal extracts are
studied as potential feed additives in their nutrition [11–14]. To extend the knowledge regarding bacteriocin applications in rabbit farms, nisin, gallidermin, and enterocins 4231, 7420, EF55, A/P and M were supplemented to the rabbits’ diet alone or in combination with phyto-additives [15–24]. Most of these studies present the bacteriocins/enterocins’ effect on growth performance, intestinal microbial composition and enzymatic activity, gut morphology and the immune response of rabbits. The monitoring of changes in rabbit meat properties due to bacteriocins/enterocins applications is further limited [25–29]. Plants (whole plants, leaves, seeds as feedstuff) and their extracts (applied as additives) are often used in rabbit nutrition due to their ability to stimulate appetite, digestion, immunity and physiological processes, as well as their strong antimicrobial, anti-inflammatory and antioxidant effects. Among herbal extracts and phyto-additives, fennel, thyme, rosemary, sage, and oregano leaves, seeds and extracts are the most often supplemented to diets of rabbit meat, and meat products are enriched with them. There is also a growing interest in sage (Salvia officinalis) plants, seeds and extracts for use in animal feeding due to their oil content, which is a source of polyunsaturated fatty acid (PUFA-linoleic and α-linolenic acid). Dietary administration with sage and its extracts/by-products could increase the PUFA content of animal products (eggs, meat [14]). Several previous studies demonstrated that a combined application of enterocins and sage extract did not have a negative influence on the characteristics of rabbit carcasses [25,27]. Moving forward from these results, the objectives of this in vivo study were to determine the effects of non-commercial Ent 7420 and sage extract administration in drinking water, both separately and in combination, on the physico-chemical parameters and mineral composition of rabbit meat.

2. Materials and Methods

2.1. Animals, Experiment Design and Diet

The experiment was performed in cooperation with our colleagues at the National Agricultural and Food Centre (NAFC, Lužianky-Nitra, Slovakia). All applicable international, national and/or institutional guidelines for animal care were followed appropriately, and the experimental protocol was approved by the Institutional Ethic Committee, and the State Veterinary and Food Administration of the Slovak Republic (permission code: SK CH 17016 and SK U 18016).

A total of 96 weaned Hyla breed male rabbits, aged 35 days (average live weight 767.2 g ± 17.5) were divided into four groups (n = 24), each consisting of 6 replicates (1 replicate/4 rabbits/2 cages, 1 cage/2 animals). Rabbits were housed in standard cages (61 cm × 34 cm × 33 cm) in a closed building equipped with heating and a forced ventilation system, which allowed the environmental temperature to be adjusted within the range of 20 ± 4 °C and to a relative humidity (70 ± 5%). The photoperiod was 16L:8D. The animals were fed with a commercial pelleted basal diet for growing rabbits (Table 1) with access to feed and water ad libitum during the experiment.

The rabbits in group E were administered enterocin Ent 7420 (a dose of 50 µL/animal/day, with activity of 25,600 AU/mL, from day 0/1 to day 21) in their drinking water, through nipple drinkers. The semi-purified Ent 7420 was prepared according to Simonová and Lauková [29], and its activity was tested using the agar spot test according to De Vuyst et al. [31] against the principal indicator strain E. avium EA5 (isolated from piglet in our laboratory) and expressed in arbitrary units per mL (AU/mL). The rabbits in group S received sage plant extract (Salvia officinalis extract containing of 24% thujone, 18% borneol, 15% cineole; Calendula, Nová Lúbovňa, Slovakia) in their drinking water at a dose of 10 µL/animal/day. The animals in the E + S groups were administered the combination of Ent 7420 and sage extract. Based on our previous experiments, showing that these additives could be dissolved in distilled water [31], the additives were first applied 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 additives being applied to their drinking water, and they were fed a commercial diet. The experiment lasted for 42 days.
2.2. Slaughtering, Physico-Chemical and Mineral Analysis

At days 21 and 42, 6 rabbits from each group (n = 6, 1 rabbit/1 replicate) were selected based on daily weight measurement to ensure similar weight of animals (day 21; average live weight: 1697.5 g ± 123.5; day 42; average live weight: 2595.2 g ± 169.8). Rabbits were slaughtered after electro-stunning (50 Hz, 0.3 A/rabbit/4 s) in an experimental slaughterhouse by cutting the carotid and jugular veins, and they quickly bled out. *Longissimus thoracis et lumborum* (LTL) was separated by removing the skin, fat and connective tissue, before being chilled and stored 24 h at 4 °C until physicochemical and mineral content analysis started.

**Table 1.** Composition and ingredients of the basal diet.

| Feed Ingredients (%) | Chemical Composition, Minerals and Vitamins (g/kg Feed) |
|-----------------------|---------------------------------------------------------|
| Dehydrated lucerne meal | 36.0 | Crude protein (N×6.25) | 175.0 |
| Extracted sunflower meal | 5.5 | Crude fiber | 188.3 |
| Oats | 13.0 | Fat | 32.0 |
| Wheat bran | 9.0 | Ash | 66.40 |
| Dry malting sprouts | 15.0 | Organic matter | 847.5 |
| Extracted rapeseed meal | 5.5 | Acid detergent fiber (ADF) | 185.1 |
| Barley | 8.0 | Neutral detergent fiber (NDF) | 315.5 |
| DDGS | 5.0 | Lignine | 42.3 |
| Sodium chloride | 0.3 | Hemicellulose | 148.5 |
| Premix minerals 1 | 1.7 | Cellulose | 148.8 |
| Limestone | 1.0 | Starch | 127.2 |
| | | Calcium | 7.5 |
| | | Phosphorus | 5.9 |
| | | Metabolic energy (MJ/kg) | 10.3 |

Abbreviations: DDGS, dried distillated grains with solubles. 1 Premix contains per kg: Calcium 6.73 g; phosphorous 4.13 g; magnesium 1.90 g; sodium 1.36 g; potassium 11.21 g; iron 0.36 g; zinc 0.13 g; copper 0.03 g; and selenium 0.2 mg. Vitamin mixture provided per kg of diet: Vitamin A 1,500,000 IU; Vitamin D3 125,000 IU; Vitamin E 5000 mg; Vitamin B1 100 mg; Vitamin B2 500 mg; Vitamin B6 200 mg; Vitamin B12 0.01 mg; Vitamin K3 0.5 mg; biotin 10 mg; folic acid 25 mg; nicotinic acid 4000 mg; and choline chloride 100,000 mg. The metabolizable energy content was calculated using the equation of Wiseman et al. [30].

The ultimate pH was determined at 24 h post mortem using a Radelkis OP-109 measuring device (Jenway, Felsted, UK) with a combined electrode penetrating 3 mm into samples. Color measurements were taken on the LTL surface of the carcass at 24 h after bleeding. Color characteristics were expressed using the CIE L*a*b* system (lightness—L*, 0: black and 100: white), (redness and greenness—a*; yellowness and blueness—b*) with a Lab Miniscan (HunterLab, Reston, VA, USA) according to the CIE Lab standards. Lightness measurements at room temperature were also taken. Total water, protein and fat contents were estimated using a FoodScaneTM-Meat Analyser (FOSS Analytical, Hilleore, Denmark) by an FT IR method (Fourier Transform infrared Spectroscopy); expressed in g/100. From these values, the energy value was calculated (EC (kJ/100 g) = 16.75 × Protein content (g/100 g) + 37.68 × Fat content (g/100 g)); Strmiska et al. [32]. Water-holding capacity (WHC) was determined by compress method at constant pressure [33]. The analyzed samples (0.3 g in weight) were placed on filter papers (Schleicher and Shuell No. 2040B, Dassel, Germany) with previously weighed tweezers. Together with the papers, samples were sandwiched between Plexiglas plates and then subjected to a pressure of 5 kg for 5 min. The results were calculated from the difference in weight between the slips with the aspirating spot and the pure filter paper. The ash content was determined by mineralization of the samples at 550 °C according to STN 570185.

Macro and micro element analysis samples wereashed at 550 °C, and the ash was dissolved in 10 mL of HCL (1:3). Minerals were determined by AAS iCE 3000 (Thermo Fisher Scientific, Waltham, MA, USA). The phosphorus content was determined by the molybdovanadate reagent on Camspec M501 (Spectronic Campes Ltd., Leeds, UK).
2.3. Statistical Analysis

Treatment effects on the meat parameters were analyzed using a two-way analysis of variance (ANOVA), followed by a Bonferroni post hoc test for pair-wise comparisons, where appropriate. Fixed effects for the model included period and treatment and the interaction between them. Random terms included cage. The statistical model included the effects of period and treatment and their interactions. Data are expressed as means and standard deviations (SD). Mean values within the same row with different superscripts indicate a significant difference \( p \leq 0.05 \). All statistical analyses were performed using GraphPad Prism statistical software (GraphPad Prism version 6.0, GraphPad Software, San Diego, CA, USA).

3. Results

The physico-chemical characteristics of the LTL are shown in Table 2. Only the time effect was noted on the meat energy value. No negative effect of tested additives was noted on the analyzed parameters. Reduced levels (although not significant) of pH24, WHC and energy values were found in all experimental groups compared to control data, while water content slightly increased.

Table 2. The effect of Ent 7420 (E), sage extract (S) and their combinative (E + S) application on the meat physico-chemical characteristics of rabbits Longissimus thoracis and lumborum (LTL; mean ± SD).

| Parameter                  | Day of Experiment | E      | S      | E + S   | C      | Significance of Effects |
|----------------------------|-------------------|--------|--------|---------|--------|-------------------------|
| pH 24 h after killing      | 21                | 5.82 ± 0.06 | 5.88 ± 0.01 | 5.84 ± 0.09 | 5.90 ± 0.01 | 1.0000 0.9741 1.0000 |
|                           | 42                | 5.66 ± 0.06 | 5.67 ± 0.08 | 5.67 ± 0.09 | 5.73 ± 0.02 | 1.0000 0.9741 1.0000 |
| Water content (g/100 g)   | 21                | 75.17 ± 0.11 | 75.03 ± 0.06 | 75.20 ± 0.26 | 74.97 ± 0.47 | 1.0000 0.9741 1.0000 |
|                           | 42                | 75.30 ± 0.10 | 75.63 ± 0.35 | 75.33 ± 0.29 | 75.47 ± 0.38 | 1.0000 0.9741 1.0000 |
| Protein content           | 21                | 22.50 ± 0.00 | 22.63 ± 0.06 | 22.33 ± 0.25 | 22.63 ± 0.38 | 1.0000 0.9741 1.0000 |
| (g/100 g)                 | 42                | 22.27 ± 0.06 | 21.97 ± 0.15 | 22.60 ± 0.30 | 22.40 ± 0.57 | 1.0000 0.9741 1.0000 |
| Fat content (g/100 g)     | 21                | 1.33 ± 0.12 | 1.33 ± 0.06 | 1.47 ± 0.21 | 1.40 ± 0.17 | 1.0000 0.9741 1.0000 |
|                           | 42                | 1.43 ± 0.15 | 1.40 ± 0.20 | 1.07 ± 0.15 | 1.23 ± 0.06 | 1.0000 0.9741 1.0000 |
| Ash content (g/100 g)     | 21                | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.0000 1.0000 1.0000 |
|                           | 42                | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.0000 1.0000 1.0000 |
| L* (lightness)            | 21                | 50.06 ± 3.30 | 47.63 ± 2.39 | 50.48 ± 2.39 | 49.97 ± 5.46 | 0.9996 0.9985 0.9983 |
|                           | 42                | 48.65 ± 5.75 | 51.42 ± 1.31 | 46.06 ± 5.74 | 51.88 ± 3.24 | 0.9996 0.9985 0.9983 |
| a* (redness)              | 21                | 0.72 ± 0.60 | 2.82 ± 1.57 | 3.63 ± 0.35 | 1.18 ± 0.97 | 0.4560 0.0809 0.0693 |
|                           | 42                | 1.64 ± 0.86 | 0.55 ± 0.20 | 0.93 ± 0.43 | 1.30 ± 0.40 | 0.4560 0.0809 0.0693 |
| b* (yellowness)           | 21                | 7.39 ± 2.23 | 7.80 ± 1.70 | 6.86 ± 1.14 | 7.25 ± 0.91 | 0.4954 0.4411 0.9302 |
|                           | 42                | 7.27 ± 0.12 | 7.26 ± 0.38 | 5.80 ± 1.09 | 7.14 ± 0.94 | 0.4954 0.4411 0.9302 |
| Water-holding capacity (g/100 g) | 21              | 36.44 ± 0.74 | 36.91 ± 1.81 | 37.27 ± 1.91 | 34.47 ± 4.21 | 0.4967 0.5436 0.2302 |
|                           | 42                | 35.86 ± 1.56 | 37.08 ± 1.07 | 34.04 ± 1.93 | 36.09 ± 3.50 | 0.4967 0.5436 0.2302 |
| Energy value (kJ/100 g)   | 21                | 427.12 ± 4.35 | 429.35 ± 1.89 | 428.89 ± 8.10 | 431.86 ± 10.70 | 0.8339 0.0060 0.3941 |
|                           | 42                | 426.97 ± 4.86 | 420.69 ± 10.06 | 418.74 ± 6.28 | 420.00 ± 7.14 | 0.8339 0.0060 0.3941 |

Ent 7420 and sage treatment reduced calcium (day 21; E vs. E + S; \( p < 0.05 \); E, S vs. C; \( p < 0.01 \); day 42; S vs. E, E + S, C; \( p < 0.01 \); E + S vs. S, C; \( p < 0.01 \); day 42; E vs. E + S, C; \( p < 0.01 \) and manganese (day 21; E + S vs. E, S, C; \( p < 0.01 \); E, S vs. C; \( p < 0.01 \); day 42; E, S, E + S, C; \( p < 0.01 \) contents and significantly elevated iron levels (day 21; E vs. S; \( p < 0.001 \); E vs. E + S, C; \( p < 0.01 \); Table 3). While sage application mostly influenced the macro minerals, with the highest phosphorus (S), magnesium and natrium (E + S) levels, Ent 7420 (E) application elevated potassium levels, while iron and zinc and reduced the calcium and copper concentrations, demonstrating the highest/lowest values.
Table 3. The effect of Ent 7420 (E), sage extract (S) and their combinative (E + S) application on the mineral content of rabbits Longissimus thoracis and lumborum (LTL; mean ± SD).

| Parameter     | Day of Experiment | E          | S          | E + S       | C          | Significance of Effects |
|---------------|-------------------|------------|------------|------------|------------|------------------------|
|               |                   | E + S      | C          | Treatment  | Time       | Interaction            |
| Calcium (mg/100 g) | 21                | 6.20 ± 0.01 a | 8.20 ± 0.04 b | 11.30 ± 0.04 b | 12.80 ± 0.01 c | <0.0001                |
|               | 42                | 8.13 ± 0.01 a | 9.10 ± 0.01 b | 7.73 ± 0.01 c | 16.77 ± 0.04 d | <0.0001                |
| Phosphorus (mg/100 g) | 21               | 201.93 ± 0.44 | 228.47 ± 0.12 | 218.77 ± 0.06 | 206.20 ± 0.18 | 0.0126                |
|               | 42                | 221.03 ± 0.17 a | 215.65 ± 0.26 b | 195.33 ± 0.04 a | 225.33 ± 0.09 b | 0.6447                |
| Magnesium (mg/100 g) | 21               | 25.10 ± 0.01 | 25.37 ± 0.01 | 25.40 ± 0.01 | 24.77 ± 0.01 | <0.0001                |
|               | 42                | 26.73 ± 0.01 | 26.30 ± 0.01 | 27.07 ± 0.01 | 26.13 ± 0.01 | <0.0001                |
| Natrium (mg/100 g) | 21               | 31.63 ± 0.02 | 30.53 ± 0.02 | 34.10 ± 0.03 | 29.27 ± 0.02 | 1.0000                |
|               | 42                | 29.07 ± 0.02 | 29.73 ± 0.01 | 29.17 ± 0.01 | 29.50 ± 0.01 | 0.1928                |
| Potassium (mg/100 g) | 21               | 413.67 ± 0.14 | 400.47 ± 0.03 | 396.67 ± 0.06 | 401.77 ± 0.22 | 0.6994                |
|               | 42                | 411.53 ± 0.16 | 410.73 ± 0.10 | 407.43 ± 0.03 | 406.30 ± 0.11 | 0.2693                |
| Iron (mg/100 g) | 21                | 0.579 ± 0.035 a | 0.341 ± 0.124 b | 0.465 ± 0.092 b | 0.365 ± 0.146 b | 0.0491                |
|               | 42                | 0.481 ± 0.035 | 0.410 ± 0.081 | 0.355 ± 0.024 | 0.465 ± 0.053 | 0.0088                |
| Manganese (mg/100 g) | 21               | 0.064 ± 0.004 a | 0.061 ± 0.009 a | 0.066 ± 0.025 b | 0.084 ± 0.010 a | <0.0001                |
|               | 42                | 0.029 ± 0.012 a | 0.027 ± 0.051 a | 0.018 ± 0.007 a | 0.082 ± 0.048 b | 0.0024                |
| Zinc (mg/100 g) | 21                | 1.350 ± 0.244 | 1.123 ± 0.141 | 1.216 ± 0.143 | 1.043 ± 0.190 | 0.2357                |
|               | 42                | 1.779 ± 0.517 a | 1.647 ± 0.504 a | 1.989 ± 0.485 a | 1.190 ± 0.131 b | 0.0126                |
| Copper (mg/100 g) | 21                | 0.117 ± 0.017 a | 0.119 ± 0.015 a | 0.195 ± 0.025 b | 0.120 ± 0.074 a | 0.0026                |
|               | 42                | 0.208 ± 0.034 a | 0.109 ± 0.003 b | 0.138 ± 0.013 b | 0.200 ± 0.066 c | 0.0824                |

a, b, c = mean values marked with different letters differ significantly at p ≤ 0.05.

4. Discussion

The Ent 7420 and sage application did not negatively influence the physico-chemical properties of rabbit meat, similarly to previous results achieved through bioactive compounds, such as the supplementation of bacteriocins, herbal extracts and beneficial strains to rabbits [26–28,34–36]. Meineri et al. [37] and Rotolo et al. [38] also reported the adverse effects of chia seeds and dried leaves on the traits of rabbit meat quality. However, no significant changes within the tested parameters were determined. The pH and WHC decreased with increasing age, similar to findings presented by Pogány Simonová et al. [34,35] and Koziol et al. [39]. The pH (acidity) of rabbit meat is an essential parameter, indicating its shelf life and preventing the microbial growth (bacteriostatic effect of low pH), technological usability and quality of the rabbit meat, which also depends on many factors, such as stress during transport and slaughter, the extent of deblooding and muscle type. Although higher pH values of LTL samples were found when compared with our previous studies, they were still under or at the upper limit of bibliographic values [40,41] and did not negatively influence the positive quality of meat. There is a relation between pH and WHC (increase in pH, increase in WHC); this finding was confirmed during the beneficial E. faecium CCM7420 and EF9a strains administration to rabbits [35,36], while enterocins and sage extract supplementation [27,28,34] showed the opposite effect (decreased pH, increased WHC), as was also found in the recent experiment with Ent7420 and sage addition. Lower energy values (but within the range of bibliographic values; [1,40]) of rabbit meat were found compared to other enterocins and sage extract applications [27,28,34], but these values were still higher than those after beneficial E. faecium strain supplementation [35,36].

Rabbit meat mineral content has a great variability. Most of tested minerals (except calcium) were measured at lower levels than previously presented by Dalle Zotte and Szendrő [1], Hermida et al. [42], and Nistor et al. [43]. Going forward from the increased levels of most tested minerals in experimental groups, we hypothesize an enhanced nutrient uptake from the intestine, and better mineral inclusion in rabbit meat. It was also interesting to find out that the combined administration of Ent 7420 and sage affected some minerals, such as phosphorus (S), iron, zinc (E), calcium and copper (E, S) in the opposite way when compared to their separate applications. These findings suggest a more antagonistic effect of tested compounds in the case of phosphorus (increased in E + S compared to lower levels in E and decreased compared to higher level in S), iron and zinc (reduced in E + S compared to elevated concentration in E and increased compared to lower S level). Regarding the copper value, we assumed the synergistic effect of sage and Ent 7420 (higher level in E + S compared to E, S and C). Nevertheless, further experiments are needed to determine...
and/or confirm in more detail if there is any other synergistic or antagonistic effect of both additives on the tested minerals. It is known that probiotics and prebiotics can affect intestinal mineral absorption by releasing bone-modulating factors such as phytoestrogens from food [44]. This finding was also noted in this study regarding the bone minerals, but mostly in the highest calcium level during the combination of Ent 7240 and sage extract in rabbits which confirmed the supportive effect of Ent 7420 on higher phytoestrogens release from sage enriched feed due to improved intestinal microbial environment. However, this synergistic interaction of tested bioactive compounds on meat calcium level was noted in E + S. The reduced Ca content compared to control Ca value did not confirm the positive effects of phyto-estrogenic compounds in sage on Ca intestinal absorption (through estrogen receptors within intestinal cells) and/or remodulation of serum Ca levels, as was previously recorded by Pogány Simonová et al. [26]. Probiotics can increase mineral solubility via an increased bacterial production of short-chain fatty acids (SCFA), enlarge the absorption surface by promoting proliferation of enterocytes mediated by bacterial fermentation products, improve gut health, and increase the expression of calcium-binding proteins, mostly elevating calcium and magnesium absorption [44]. Another way to enhance mineral absorption due to ionization and passive diffusion, is the acidic environment as a result of a higher lactic acid formation [45]. Beneficial gut bacteria, also enhanced/optimized by natural feed additives may improve the availability and absorption of polyvalent cations, such as calcium, phosphorus, magnesium, zinc and iron due to optimum pH conditions for enzymatic phytate degradation and reduction. This finding was repeatedly confirmed after Enterococcus faecium CCM7420 and CCM8558 probiotic strains dietary inclusion in rabbits, when phosphorus, iron and zinc concentrations in meat were increased compared to untreated animals [35,46]. Bacteriocins in meaning postbiotics (metabolites of beneficial bacteria) can balance/improve the host microbiome in favor of lactic acid bacteria because of their antimicrobial activity, which inhibits the growth of enteropathogenic bacteria, and thus ensures a higher lactic acid production. This may be another explanation of higher iron and zinc intestinal absorption and the inclusion of rabbit meat, mostly as a result of the higher iron level in meat samples from rabbits receiving Ent 7420. This hypothesis is also confirmed by the results showing an increase in phosphorus, iron and zinc after enterocin M application to rabbits [46].

In the case of iron content in food, it is important to know the respective amounts of ferrous (heme; Fe$^{2+}$) iron, sources from meat, liver and meat products, and ferric (non-heme; Fe$^{3+}$) iron, source from legumes, cereals, vegetables and fruits, with a dietary transformation between these two states. Heme iron is the most bioavailable form of iron, due to its coordination with a porphyrin ring hidden inside a globular protein, and this arrangement protects iron from oxidation and insoluble precipitates forming in the intestine, which promote its bioavailability [47]. Although there are only a few studies concerning the probiotic and postbiotic/bacteriocin effect on rabbit meat, the achieved results show an improvement of rabbit meat quality due to its higher iron content. Contrary to us, Shah et al. [48] observed decreased iron and zinc content in rabbit hind leg samples after microbial fermented feed utilization. On the other hand, these authors noted lower copper and manganese levels, similar to our present and previous results [27,35,46]. Diet supplementation with sage extract/chia seeds was shown to be effective in improving rabbit meat nutritional quality, focusing on its fatty acid profile, and this meat can be considered as a functional food [1]. Because there are only a few studies regarding the effect of sage extract on rabbit meat minerals, we can only assume the activity of phenolic compounds in increasing bone mineral content and bioavailability for iron [49]. Further research will be necessary to clarify the effect of sage bioactive components on minerals absorption and its inclusion in meat.

5. Conclusions

It seems that diet supplementation with Ent 7420 and sage extract was effective in improving the meat mineral profile. While Ent 7420 significantly elevated the iron
content and increased zinc and potassium levels, sage extract beneficially influenced the phosphorus and zinc concentrations of rabbit meat. Reduced calcium and manganese levels were found after Ent 7420 and sage application. Both additives in combination also increased phosphorus, iron, zinc and copper concentrations, without any adverse effects on the physico-chemical properties of rabbit meat. We conclude that diet supplementation, mainly with Ent 7420, can enhance the nutritional quality of rabbit meat.

Author Contributions: Conceptualization, M.P.S. and A.L.; methodology, I.C.; validation, M.P.S.; investigation, M.P.S., I.C. and A.L.; resources, M.P.S.; data curation, I.C. and M.P.S.; writing—original draft preparation, M.P.S.; writing—review and editing, M.P.S.; visualization, M.P.S.; supervision, M.P.S. and A.L.; project administration, M.P.S.; funding acquisition, M.P.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Scientific Grant Agency of the Ministry for Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences VEGA, grant number 2/0055/21.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the State Veterinary and Food Administration of the Slovak Republic on 1 December 2016 (approval numbers SK CH 17016 and SK U 18016).

Data Availability Statement: Data are available upon reasonable request to the corresponding author.

Acknowledgments: We are grateful to P. Jerga for his skillful technical assistance and to J. Pecho for slaughtering.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Dalle Zotte, A.; Szendrő, Z. The role of rabbit meat as functional food. Meat Sci. 2011, 88, 319–331. [CrossRef] [PubMed]
2. Yang, S.-C.; Lin, C.-H.; Sung, C.T.; Fang, J.-Y. Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. Front. Microbiol. 2014, 5, 241. [PubMed]
3. Bemena, L.D.; Mohamed, L.A.; Fernandes, A.M.; Lee, B.H. Applications of bacteriocins in food, livestock health and medicine. Int. J. Curr. Microbiol. Appl. Sci. 2014, 3, 924–949.
4. Hernández-González, J.C.; Martínez-Tapia, A.; Lazcano-Hernández, G.; García-Pérez, B.E.; Castrejón-Jiménez, N.S. Bacteriocins from lactic acid bacteria. A powerful alternative as antimicrobials, probiotics, and immunomodulators in veterinary medicine. Animals 2021, 11, 979. [CrossRef] [PubMed]
5. Hernández-González, F. Applications of bacteriocins in livestock. Curr. Issues Intest. Microbiol. 2007, 8, 15–24.
6. Vieco-Saiz, N.; Belguesmia, Y.; Raspoet, R.; Auclair, E.; Gancel, F.; Kempf, I.; Drider, D. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Front. Microbiol. 2019, 10, 57. [CrossRef]
7. Strompfová, V.; Marciniaková, M.; Simonová, M.; Gancarčíková, S.; Jonecková, Z.; Sciráňková, L.; Koščová, J.; Buleca, V.; Čobanová, K.; Lauková, A. Enterococcus faecium EK13—An enterocin A-producing strain with probiotic character and its effect in piglets. Anaerobe 2006, 12, 242–248. [CrossRef]
8. Lauková, A.; Kandričková, A.; Šcerbová, J. Use of bacteriocin-producing, probiotic strain Enterococcus faecium AL41 to control intestinal microbiota in farm ostriches. Lett. Appl. Microbiol. 2015, 60, 531–535. [CrossRef]
9. Lauková, A.; Guba, P.; Nemcová, R.; Vasiliková, Z. Reduction of Salmonella in gnotobiotic Japanese quails caused by the enterocin A-producing EK13 strain of Enterococcus faecium. Vet. Res. Commun. 2003, 27, 275–280. [CrossRef]
10. Lauková, A.; Guba, P.; Nemcová, R.; Mareková, M. Inhibition of Salmonella enterica serovar Dusseldorf by enterocin A in gnotobiotic Japanese quails. Vet. Med. 2004, 49, 47–51. [CrossRef]
11. Kalma, R.P.; Patel, V.K.; Joshi, A.; Umatiya, R.V.; Parmar, K.N.; Damor, S.V.; Chauhan, H.D.; Srivastava, A.K.; Sharma, H.A. Probiotic supplementation in rabbit: A review. Int. J. Agric. Sci. 2016, 8, 2811–2815.
12. Mancini, S.; Paci, G. Probiotics in rabbit farming: Growth performance, health status, and meat quality. Animals 2021, 11, 3388. [CrossRef] [PubMed]
13. Dalle Zotte, A.; Celia, C.; Szendrő, Z. Herbs and spices inclusion as feedstuff or additive in growing rabbit diet and as additive in rabbit meat: A review. Livest. Sci. 2016, 189, 82–90. [CrossRef]
15. Szabóvá, R.; Lauková, A.; Chrastínová, L.; Stropová, V.; Pogány Simonová, M.; Vasilková, Z.; Čobanová, K.; Plachá, I.; Chrenková, M. Effect of combined administration of enterocin 4231 and sage in rabbits. Pol. J. Vet. Sci. 2011, 14, 359–366. [CrossRef]

16. Lauková, A.; Chrastínová, L.; Pogány Simonová, M.; Stropová, V.; Plachá, I.; Čobanová, K.; Formelová, Z.; Chrenková, M.; Ondruška, L. Enterococcus faecium AL 41: Its enterocin M and their beneficial use in rabbits husbandry. Probiotics Antimicrob. Proteins 2012, 4, 243–249. [CrossRef]

17. Lauková, A.; Chrastínová, L.; Plachá, I.; Kandričáková, A.; Szabóvá, R.; Stropová, V.; Chrenková, M.; Čobanová, K.; Žitňan, R. Beneficial effect of lantibiotic nisin in rabbit husbandry. Probiotics Antimicrob. Proteins 2014, 6, 41–46. [CrossRef]

18. Lauková, A.; Pogány Simonová, M.; Chrastínová, L.; Kandričáková, A.; Ščerbová, J.; Plachá, I.; Čobanová, K.; Formelová, Z.; Ondruška, L.; Strkolková, G.; et al. Beneficial effect of bacteriocin-producing strain Enterococcus durans ED 26E/7 in model experiment using broiler rabbits. Czech J. Anim. Sci. 2017, 62, 168–177. [CrossRef]

19. Lauková, A.; Pogány Simonová, M.; Chrastínová, L.; Gancarčíková, S.; Kandričáková, A.; Plachá, I.; Čobanová, K.; Formelová, Z.; Ondruška, L.; Ščerbová, J.; et al. Assessment of lantibiotic type bacteriocin gallidermin application in model experiment with broiler rabbits. Int. J. Anim. Sci. 2018, 2, 1028.

20. Lauková, A.; Chrastínová, L.; Plachá, I.; Szabóvá, R.; Kandričáková, A.; Pogány Simonová, M.; Formelová, Z.; Ondruška, L.; Goldová, M.; Chrenková, M.; et al. Enterocin 55 produced by non rabbit-derived strain Enterococcus faecium EF55 in relation with microbiota and selected parameters in broiler rabbits. Int. J. Environ. Agric. Res. 2017, 3, 45–52.

21. Pogány Simonová, M.; Lauková, A.; Chrastínová, L.; Stropová, V.; Faix, Š.; Vasilková, Z.; Ondruška, L.; Jürčík, R.; Rafay, J. Enterococcus faecium CCM7420, bacteriocin PPB CCM7420 and their effect in the digestive tract of rabbits. Czech J. Anim. Sci. 2009, 54, 376–386. [CrossRef]

22. Pogány Simonová, M.; Lauková, A.; Plachá, I.; Čobanová, K.; Stropová, V.; Szabóvá, R.; Chrastínová, L. Can enterocins affect phagocytosis and glutathione peroxidase in rabbits? Cent. Eur. J. Biol. 2013, 8, 730–734.

23. Pogány Simonová, M.; Chrastínová, L.; Lauková, A. Autochtonous strain Enterococcus faecium EF2019(CCM7420), its bacteriocin and their beneficial effects in broiler rabbits: A review. Animals 2020, 10, 1188. [CrossRef]

24. Pogány Simonová, M.; Chrastínová, L.; Kandričáková, A.; Gancarčíková, S.; Bino, E.; Plachá, I.; Ščerbová, J.; Stropová, V.; Žitňan, R.; Lauková, A. Can have enterocin M in combination with sage beneficial effect on microbiota, blood biochemistry, phagocytic activity and jejunal morphometry in broiler rabbits? Animals 2020, 10, 115. [CrossRef] [PubMed]

25. Pogány Simonová, M.; Chrastínová, L.; Lauková, A. Enterocin Ent7420 and sage application as feed additives for broiler rabbits to improve meat carcass parameters and amino acid profile. Meat Sci. 2022, 183, 108656. [CrossRef] [PubMed]

26. Pogány Simonová, M.; Chrastínová, L.; Lauková, A. Lantibiotic nisin applied in broiler rabbits and its effect on the growth performance and carcass quality. Probiotics Antimicrob. Proteins 2019, 11, 1414–1417. [CrossRef] [PubMed]

27. Pogány Simonová, M.; Chrastínová, L.; Lauková, A.; Formelová, Z.; Kandričáková, A.; Bino, E.; Lauková, A. Benefit of enterocin M and sage combination on physico-chemical traits, fatty acid, amino acid, and mineral content of rabbit meat. Probiotics Antimicrob. Proteins 2020, 12, 1235–1245. [CrossRef]

28. Chrastínová, L.; Lauková, A.; Chrenková, M.; Polačiková, M.; Formelová, Z.; Kandričáková, A.; Glatzová, E.; Ščerbová, J.; Bučko, O.; Ondruška, L.; et al. Effects of enterocin M and durancin ED26E/7 substances applied in drinking water on the selected carcass characteristics and meat quality of broiler rabbits. Arch. Zoootech. 2018, 21, 5–17.

29. Chrastínová, L.; Chrenková, M.; Formelová, Z.; Lauková, A.; Pogány Simonová, M.; Rajsík, M.; Polačiková, M.; Plachá, I.; Bačová, K.; Bučko, O.; et al. Use of enterocin M substance applied in drinking water and natural zeolite as dietary supplements for growing rabbits. Slovak J. Anim. Sci. 2020, 53, 12–18.

30. Simonová, M.; Lauková, A. Bacteriocin activity of enterococci from rabbits. Vet. Res. Comm. 2007, 31, 143–152. [CrossRef]

31. Wiseman, J.; Edmunds, B.K.; Shepperson, N. The apparent metabolisable energy of sunflower oil meal and sunflower oil acid for broiler chickens. Anim. Feed Sci. Technol. 1992, 36, 41–45. [CrossRef]

32. De Vuyts, L.; Callewaert, R.; Pot, B. Characterization of the antagonistic activity of Lactobacillus amylovorus DCE471 and large-scale isolation of its bacteriocin amylovorin L471. Syst. Appl. Microbiol. 1996, 9, 9–20. [CrossRef]

33. Strmiska, F.; Holčíková, K.; Simonová, E.; Mrázová, E.; Hodeková, J.; Votášsková, A.; Pristašová, M.; Strmiska, J.; Strmisková, G.; Kruparovičová, M.; et al. Požitivitnost Tabulky I—Potravinárské Surowiny, Food Tables I—Primary Foods; Výskumný Ústav Potravinárský (Food Research Institute): Bratislava, Slovakia; 1998; p. 189. (In Slovak with English Summary and Register).

34. Hašek, O.; Palanská, O. Determination of water holding capacity in meat by instruments at constant pressure (in Slovak). Poultry Ind. 1976, 8, 228–233.

35. Pogány Simonová, M.; Chrastínová, L.; Mojto, J.; Lauková, A.; Szabóvá, R.; Rafay, J. Quality of rabbit meat and phyto-additives. Czech J. Food Sci. 2010, 28, 161–167. [CrossRef]

36. Pogány Simonová, M.; Chrastínová, L.; Lauková, A. Dietary supplementation of a bacteriocinogenic and probiotic strain of Enterococcus faecium CCM7420 and its effect on the mineral content and quality of Musculus longissimus dorsi in rabbits. Anim. Prod. Sci. 2016, 56, 2140–2145. [CrossRef]

37. Pogány Simonová, M.; Chrastínová, L.; Lauková, A. Effect of beneficial strain Enterococcus faecium EF9a isolated from Pannon White rabbit on growth performance and meat quality of rabbits. Ital. J. Anim. Sci. 2020, 19, 650–655. [CrossRef]

38. Meineri, G.; Cornale, P.; Tassone, S.; Peiretti, P.G. Effects of Chia (Salvia hispanica L.) seed supplementation on rabbit meat quality, oxidative stability and sensory traits. Ital. J. Anim. Sci. 2010, 9, 45–49. [CrossRef]
39. Rotolo, L.; Gai, F.; Nicola, S.; Zoccarato, I.; Brugiapaglia, A.; Gasco, L. Dietary supplementation of oregano and sage dried leaves on performances and meat quality of rabbits. *J. Integr. Agric.* **2013**, *12*, 1937–1945. [CrossRef]
40. Kozioł, K.; Maj, D.; Bieniek, J. Changes in the color and pH of rabbit meat in the aging process. *Med. Weter.* **2015**, *71*, 104–108.
41. Dalle Zotte, A. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livest. Prod. Sci.* **2002**, *75*, 11–32. [CrossRef]
42. Pla, M.; Guerrero, L.; Guardia, D.; Olive, M.A.; Blasco, A. Carcass characteristics and meat quality of rabbit lines selected for different objectives: I. Between lines comparison. *Livest. Prod. Sci.* **1998**, *54*, 115–123. [CrossRef]
43. Hermida, M.; Gonzalez, M.; Miranda, M.; Rodriguez-Otero, J.L. Mineral analysis in rabbit meat from Galicia (NW Spain). *Meat Sci.* **2016**, *73*, 635–639. [CrossRef] [PubMed]
44. Nistor, E.; Bampidis, V.A.; Păcăla, N.; Pencea, M.; Tozer, J.; Prundeanu, H. Nutrient content of rabbit meat as compared to chicken, beef and pork meat. *J. Anim. Prod. Adv.* **2013**, *3*, 172–176. [CrossRef]
45. Scholz-Ahrens, K.E.; Adolphi, B.; Rochat, F.; Barclay, D.V.; de Vrese, M.; Açil, Y.; Schrezenmeir, J. Effects of probiotics, prebiotics, and symbiotics on mineral metabolism in ovariectomized rats—Impact of bacterial mass, intestinal absorptive area and reduction of bone turn-over. *NFS J.* **2016**, *3*, 41–50. [CrossRef]
46. Cummings, J.H.; Macfarlane, G.T.; Englyst, H.N. Prebiotic digestion and fermentation. *Am. J. Clin. Nutr.* **2011**, *73*, 415S–420S. [CrossRef]
47. Pogány Simonová, M.; Chrastinová, L.; Lauková, A. Effect of *Enterococcus faecium* AL41 (CCCM8558) and its enterocin M on the physicochemical properties and mineral content of rabbit meat. *Agriculture* **2021**, *11*, 1045. [CrossRef]
48. Verma, C.; Tapadia, K.; Soni, A.B. Determination of iron (III) in food, biological and environmental samples. *Food Chem.* **2017**, *221*, 1415–1420. [CrossRef]
49. Shah, A.A.; Liu, Z.; Qian, C.; Wu, J.; Sultana, N.; Zhong, X. Potential effect of the microbial fermented feed utilization on physicochemical traits, antioxidant enzyme and trace mineral analysis in rabbit meat. *J. Anim. Physiol. Anim. Nutr.* **2020**, *104*, 767–775. [CrossRef]