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Benefits of Enterocin M and Sage Combination on the Physico-chemical Traits, Fatty Acid, Amino Acid, and Mineral Content of Rabbit Meat

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Abstract
Higher rabbit meat consumption can be ensured by increasing of its quality due to its improved nutritional properties. The effect of enterocin M (EntM) and sage on growth performance, physico-chemical properties, fatty acid (FA), amino acid (AA), and mineral concentrations of rabbit meat was evaluated. Sixty-four rabbits (M91 meatline, both sexes) were divided into three experimental: E (EntM), S (sage), E + S (EntM + sage) groups and control group (C). The additives were administrated in drinking water during 21 days. Lower pH (E, S: $P < 0.05$; E + S: $P < 0.001$) and decrease in water content were noted in all experimental groups compared with controls (C). Higher values of lightness (L^*), yellowness (b^*), and redness (a^*; except group E) were measured. The sage administration increased the fat and protein contents ($P < 0.05$), the meat energy value (S vs. C: $P < 0.01$; S vs. E and E + S: $P < 0.05$), the concentrations of arachidonic ($P < 0.05$), eicosapentaeonic ($P < 0.05$), and oleic acids ($P < 0.01$), magnesium ($P < 0.05$), and potassium ($P < 0.01$) content. The additives did not influence the rabbit meat AA composition. The sage diet inclusion could improve the quality of rabbit meat due to its higher protein, fat, and energy contents and enhance the PUFA and mineral content of rabbit meat. Moreover, the effect of EntM on meat color parameters, FA and AA composition, has not been tested in rabbits previously.

Keywords Rabbit meat · Fatty acids · Amino acids · Minerals · Sage · Enterocin

Introduction

The healthy lifestyle and healthy food are a hot topic of humans' everyday life, containing the area of production and raw materials' processing, distribution, and consumption. Because of a great availability and variability of foods on the market, there is also great competition between producers to offer products of the highest quality products to consumers. Regarding the meat market, rabbit meat is not prevalent worldwide and its consumption is presently regressing, despite of the high nutritional and dietetic properties of rabbit meat [1], except for countries where it is considered a traditional meat species [2]. This rabbit meat-eating habits can be changed to positive side and encouraged more people to consume rabbit meat by increasenig of its quality due to its improved nutritional properties—fatty acid (FA) and amino acid (AA) profile, minerals, and vitamin content. During the last decades, studies on rabbit meat properties have been focused mainly on its chemical composition, pH, color, and also on several technical aspects—chilling and cooking loss, meat tenderness measuring of rabbit meat. The influence of probiotics, prebiotics, fatty acids, vitamins, selenium, antioxidants, and their combinations on rabbit carcass quality has been already presented in many research papers [3–10]. Concerning the rabbit meat nutritional composition and quality, FAs content of rabbit meat is also documented in many studies [1, 11–14], etc.]. On the other hand, there are only several reports dealing with rabbit meat mineral content [5, 15–19]. The AA composition of rabbit meat and changes in minerals and AA levels during natural additive treatment is also limited only to few studies [3, 4, 13, 14, 19–22].

Sage (*Salvia* spp.) is a common aromatic and medicinal plant, encompasses about 900 species of plants belonging to

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the mint family Lamiaceae (*Labiatae*), and is characterized with antioxidant, anti-inflammatory, antimicrobial, hypocholesteraemic, hypoglycaemic, and anti-mutagenic bioactivities [23]. Many *Salvia* spp. are used as herbal tea, for food flavoring, in cosmetics, perfumeries, and in the pharmaceutical industry. There is also a growing interest in sage plants, seeds, and extract use in animal feeding due to their oil content, which is a rich source of polyunsaturated fatty acids (PUFA—linoleic and α-linolenic acid). Dietary administration with sage, its extracts, and/or by-products could improve the PUFA content of animal products (eggs, meat [9]). Although the administration of herbal extracts to rabbits and their effects on rabbit meat properties have been investigated by many researchers [4, 6–12, 24–26], only a few of them have presented results concerning rabbit meat quality and composition after sage extract dietary inclusion [4, 6, 11, 25]. In recent years, except herbs and their extracts, other naturally occuring antimicrobial compounds—bacteriocins—have been preferred in animal feeding as well as in animal products for their potential safety, health benefits, and improvement of products' quality compared to synthetic preservatives. The most often used bateriocins, nisin and pediocin, have been applied also in other ecosystems: feed (silage), animals, aquacultures, and humans [27]. The individual group of bacteriocins represents enterocins, produced by different species of enteroccocci, but mainly by *Enterococcus faecium*, *E. faecalis*, *E. hirae*, *E. durans*, and *E. mundtii* [28]. While the applications of bacteriocins are expanding from food to human health, describing their properties, mode of action, and antimicrobial spectrum, after all, their "in vivo" administration in animals are often limited [29]. Moreover, the use of enterocins in rabbits is presented only in some of them and it is not so extensive [30–32]. Enterocin M (EntM) is a new enterocin (variant of enterocin P) produced by the strain *E. faecium* AL41, which was purified to homogenity and tested under in vitro conditions [33] and till now, experimentally applied only to rabbits and horses [32, 34], with beneficial effects on the growth performance and immune status of animals. Monitoring of changes in meat properties due to bacteriocins/enterocins application in rabbits is even more limited [3, 14]. These studies conclude that bacteriocin treatment did not have negative influence on the rabbit carcass characteristics. However, further investigation is needed to develop the already known facts and to achieve new and more results, concerning the physico-chemical properties of rabbit meat and its improvement through additive supplementation.

Outgoing from previously described general benefits of sage and enterocins and from the results presenting improved health status, immunity, serum parameters, intestinal microbiota, and metabolic activities through experimental application of sage and EntM in rabbits, we decided to know how these natural additives could influence the rabbit meat quality. The objective of this in vivo study was to determine the effects of EntM and sage extract administration, applied to drinking water both separately and in combination on growth performance, selected physico-chemical parameters, FA and AA composition, and mineral content of rabbit meat. To the best of our knowledge, this is the first experiment/report with rabbits in which the effect of a new, not commercial bacteriocin—EntM and sage extract on the rabbit meat properties—and its FA and AA composition have been tested, which underlines the originality of this study.

Materials and Methods

Animals and Housing

The experiment was performed at the National Agricultural and Food Centre (NAFC, Lužianky–Nitra, Slovakia). A total of 64 post-weaned rabbits (aged 35 days, both sexes) were divided into four groups (control: C and three experimental groups: E–EntM, S–sage extract, E + S–EntM in combination with sage extract, with 16 animals in each group). Rabbits were kept in standard cages (0.61 m × 0.34 cm × 0.33 m), the type D-KV-72 supplied by the Kovobel company (Domažlice, Czech Republic), two animals per cage. The rabbits of meat line M91, maternal albinotic line (crossbreed New Zealand White, Bouscat rabbit, Argente Champagnet rabbit), and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in this experiment. A cycle of 16 h light and 8 h dark was used throughout the experiment. Temperature 16 ± 4 °C and humidity 70 ± 5% were maintained throughout the experiment by heating and ventilation systems and were recorded continuously by means of a digital thermograph positioned at the same level as the cages.

Experiment Design

The animals were fed a commercial granulated diet for growing rabbits (Table 1) with access to feed and water ad libitum during the experiment. The rabbits in group E were administered EntM (prepared according to Mareková et al. [33]; a dose of 50 μL/animal/day, with activity 12,800 AU/mL, from day 1 to day 21) in their drinking water. Briefly, the semi-purified EntM was prepared by the following procedures: a 16-h culture (300 mL) of *E. faecium* CCM8558-AL41 in MRS broth (Merck KGaA, Darmstadt, Germany) was centrifuged for 30 min at 10,000×*g* in order to remove the cells. After adjusting of supernatant to pH 5.5, ammonium sulfate was gently added to the supernatant to obtain 40% (*w*/*v*) saturation, and the mixture was stirred at 21 °C for 1 h. After centrifugation at 10,000×*g* for 30 min, the resulting pellet was resuspended in 10 mmol/L sodium phosphate buffer (pH 6.5). Activity of EntM was tested using the agar spot test according

Table 1 Ingredients and composition of the commercial diet

Feed ingredients (%)	Diet	Chemical analysis, minerals and vitamins (g[a], mg[b]/kg feed)	Diet
Dehydrated lucerne meal	36.0	Dry matter[a]	882.1
Extracted sunflower meal	5.5	Crude protein[a]	164.2
Oats	13.0	Crude fiber[a]	155.5
Wheat bran	9.0	Fat[a]	33.3
Dry malting sprouts	15.0	Ash[a]	73.0
Extracted rapeseed meal	5.5	Nitrogen-free extract[a]	457.1
Barley	8.0	Organic compounds[a]	810.0
DDGS	5.0	Acid detergent fiber (ADF)[a]	191.1
Sodium chloride	0.3	Neutral detergent fiber (NDF)[a]	339.5
Premix minerals[1]	1.7	Lignine[a]	42.3
Limestone	1.0	Hemicellulose[a]	148.5
		Cellulose[a]	148.8
		Starch[a]	127.2
		Calcium[a]	6.0
		Phosphorus[a]	5.9
		Magnesium[a]	2.3
		Sodium[a]	1.7
		Potassium[a]	7.5
		Iron[b]	403.9
		Zinc[b]	166.4
		Manganese[b]	156.7
		Cuprum[b]	22.9
		Metabolic energy (MJ/kg)	11.0

DDGS dried distiller 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; 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, choline chloride 100,000 mg

to De Vuyst et al. [35] against the principal indicator strain *E. avium* EA5 (isolated from piglet feces, in our laboratory and used as a bacteriocin-sensitive indicator strain). The antimicrobial titer of entM was defined as the reciprocal of the highest twofold dilution producing a distinct inhibition of the inhibitor lawn, expressed in arbitrary units per mL (AU/mL). The rabbits in group S received sage plant extract (*Salvia officinalis* extract containing 24% thujone, 18% borneol, 15% cineole; the Calendula company, Nová Ľubovňa, Slovakia) in their drinking water at a dose of 10 μL/animal/day. The animals in the E + S were administered the combination of EntM (50 μL/animal/day, 12,800 AU/mL) strain and sage plant extract (10 μL/animal/day). The doses of additives and their manner of application form were decided on the basis of our previous in vitro studies testing the inhibitory activity of EntM and sage extract against target bacteria and an experiment with rabbit-derived bacteriocin-producing strain *E. faecium* CCM7420 [4, 36]. Based on our previous experiments, these additives can dissolved in distilled water and/or phosphate buffer [33, 36], and also, 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 additives being applied to their drinking water, and they were fed a commercial diet. Drinking water was provided through nipple drinkers. The experiment lasted for 35 days.

Performance Traits, Slaughtering and Sampling

Body weight and feed consumption were measured every week during the experiment; average daily weight gain and feed conversion were calculated mathematically. Mortality and morbidity were also recorded daily in all groups.

At days 21 and 35, eight animals from each group were randomly selected for slaughter; they were stunned with electronarcosis (90 V for 5 s) in an experimental slaughterhouse,

immediately hung by the hind legs on the processing line and quickly bled by cutting the *jugular veins* and the *carotid arteries*. After the bleeding, the *Longissimus thoracis* and *lumborum* (LTL) muscles were separated by removing the skin, fat, and connective tissue, chilled and stored 24 h at 4 °C until physico-chemical analysis started.

The ultimate pH was determined 24 h post mortem (p.m.) with a Radelkis OP-109 (Jenway, England) with a combined electrode penetrating 3 mm into the MLD. The electrical conductivity ($\mu S\ cm^{-1}$) defined as locations of muscles was evaluated using PMV 51 (Tecpro Metall GmbH, Neuss, Germany). Color measurements were taken on MLD 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*) using a Lab. Miniscan (HunterLab, Reston, VA, USA). Lightness measurements at room temperature were also taken. Total water, protein, and fat contents were estimated using an INFRATEC 1265 spectroscope (FOSS, Tecator AB, Höganäs, Sweden) and expressed in g/100 g. The near infrared transmission (NIT) principle is based on the fact that the measured sample absorbs the near infrared light at different wavelengths according to different characteristics such as fat or protein content [37]. 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) [38]]. Water holding capacity was determined by compress method at constant pressure [39]. The analyzed samples (0.3 g in weight) were placed on filter papers (Schleicher and Shuell No. 2040B, Dassel, Germany) with tweezers previously weighed. Together with the papers, samples were sandwiched between Plexiglas plates and then subjected to apressure of 5 kg for 5 min. The results were calculated from the difference in weight between the slips with aspirating spot and the pure filter paper.

The FA composition of samples LTL was determined by the method of Ouhayoun [40] by gas chromatography of fatty acid methyl ester (FAME) on GC 6890 N (Agilent Technologies (Schweiz) AG, Basel, Switzerland). Results were expressed as percentages of total FAs. FA composition varies a lot and it is expressed as share of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), PUFA, and n6/n3 index. AAs were determined in fat-free samples by ion-exchange chromatography (free AAs) and by liquid chromatography (total AAs) after acid hydrolysis in 6 M HCl and methionine and cystine (sulfur AAs) after oxidation hydrolysis, with hydrogen peroxide and formic acid. An Amino Acid Analyzator AAA 400 (Ingos s.r.o., Prague, Czech Republic) was used for separating AAs. For macro and micro element analysis, samples were ashed at 550 °C, the ash was dissolved in 10 mL of HCL (1:3), and minerals were determined by AAS iCE 3000 (Thermo Fisher Scientific, Waltham, MA, USA). Phosphorus content was determined by molybdovanadate reagent on Camspec M501 (Spectronic Campes Ltd., Leeds, United Kingdom).

Statistical Analyis

Treatment effects on tested parameters were analyzed using one-way analysis of variance (ANOVA) with Tukey post hoc test. All statistical analyses were performed using GraphPad Prism statistical software (GraphPad Prism version 6.0, GraphPad Software, San Diego, CA, USA). Differences between the mean values of the different dietary treatments were considered statistically significant at $P < 0.05$. Data are expressed as means and standard deviations of the mean (SD).

Results

Application of EntM (E) and sage extract (S) separately did not influence the growth of rabbits. The animals maintained in good health thoughout the experiment, and during combinative application (E + S), minimal mortality was recorded compared to controls (C; Table 2). On the other hand, the highest body weight gain was also noted in this group (by 0.5% compared to C). Lower feed conversion was noted in the experimental groups compared with controls (day 21), with the lowest data detected in group E.

Dietary EntM and sage supplementation affected only some carcass traits (Table 3); the lowest body and carcass weight were noted in group E ($P < 0.05$) and the highest values were measured in group S, compared to C. Moreover, carcass yield value in S was the highest among all groups, and E and E + S groups also showed a trend towards increased values of this parameter. The physico-chemichal characteristics of the LTL are shown in Table 3. The pH values measured at 24 h after slaughtering (in the range 5.57–5.74) were significantly lower in all experimental groups compared with C (E, S: $P < 0.05$; E + S: $P < 0.001$). Moreover, decreased pH value was also noted at day 35 (2 weeks after additive cessation, the end of the experiment), mainly in the E + S group ($P < 0.001$), compared to C data. In our analysis of color parameters in all experimental groups, higher values of lightness (L^*), yellowness (b^*), and redness (a^*; except group E) were measured. In all experimental groups, mainly in E + S, decrease in meat water content was noted compared to C. On the other hand, the WHC decreased only in S group, in contrast to other findings. Increased electrical conductivity (EC) was also measured by us during EntM and sage administration to rabbits, compared to untreated animals. The highest fat and the highest protein contents (S: $P < 0.05$) were measured in meat samples from rabbits receiving sage extract. Higher protein content was also found in the S + E group. The increase in meat energy value was detected in the rabbits from all experimental groups compared with C and it was also much higher

Table 2 The effect of enterocin M (E), sage extract (S), and their combinative (E + S) application on the growth performance of rabbits

	E	S	E + S	C	*P* value
Number of rabbits	(*n* = 16)	(*n* = 16)	(*n* = 16)	(*n* = 16)	
Initial live weight (35 days), g	940.6 ± 180.8	993.1 ± 300.0	1002.3 ± 162.3	1042.5 ± 315.7	0.7167
Intermediate live weight (56 days), g	1687.8 ± 171.7	1737.5 ± 347.4	1664.4 ± 170.0	1856.9 ± 361.4	0.2185
Final weight (70 days), g	2280.0 ± 174.6	2175.6 ± 259.5	2206.7 ± 164.6	2319.2 ± 164.6	0.2645
Average daily gain (g/day)	40.45	40.47	42.70	42.50	
Feed conversation ratio between 35 and 56 days of age (g/g)	2.58	2.67	2.97	3.03	
Feed conversation ratio between 56 and 77 days of age (g/g)	4.39	3.70	4.28	4.29	
Feed conversation ratio per kg gain	3.30	3.35	3.48	3.57	
Mortality (*n*)	0	0	1	0	

35–56 days of age: application of enterocin M, sage, and their combination

56–70 days of age: after the enterocin M and sage cessation

in the group S compared to C ($P < 0.01$) and E and E + S ($P < 0.05$).

The FA composition in LTL muscles is shown in Table 4. The intramuscular fat was characterized by the highest percentage of MUFAs (43.791–46.940%) and lower percentage of PUFAs (12.351–13.825%). While the total SFA concentrations were the highest in the E group among all experimental and control groups, the stearic acid level significantly decreased through EntM application (E vs. S, E + S: $P < 0.01$; E vs. C: $P < 0.001$). The concentrations of total tested MUFAs and PUFAs significantly increased in the sage-alone group (S), compared to EntM addition (MUFA: $P < 0.001$; PUFA: $P < 0.05$) and these values were numerically higher compared to C. While the total MUFA content of the LTL was significantly lower in E + S compared to C ($P < 0.01$) and to E ($P < 0.05$) groups, the opposite was true for vaccenic and eicosenic acids, which showed significantly higher levels compared to C ($P < 0.05$). The oleic acid was noted as the highest in group S (S vs. E + S: $P < 0.01$; S vs. E, C: $P = 0.0039$). As shown in Table 4, the individual dietary inclusion of sage was the most effective on the total PUFA content (13.825%; S vs. E, E + S: $P < 0.05$), with the focus on the arachidonic ($P < 0.05$) and eicosapentaeonic (EPA; $P < 0.05$) acids and only slightly (numerically) increase in the case of conjugated linoleic (CLA), linoleic and α-linoleic acids was found compared to C. The EPA levels were found in higher—but not significantly—levels also in groups E and E + S, compared to C. The highest *n*-6/*n*-3 ratio was also described in S group.

The highest protein level was noted in samples from rabbits receiving sage extract compared to groups C and M ($P < 0.05$; Table 5). Despite of this, the concentration of essential AAs was lower in all experimental groups compared to C during the additive application.

Compared with the control, the EntM and sage treatment increased the concentrations of tested minerals, except zinc and copper, in which concentrations were decreased (Table 6). The highest levels of calcium, phosphorus, magnesium ($P < 0.05$), potassium ($P < 0.01$), iron, and manganese were noted in animals receiving sage extract separately. The highest natrium value was measured in the E + S group. The EntM administration did not significantly influenced the mineral concentration of rabbit meat; only numerical increase of the phosphorus, natrium, potassium, and manganese concentrations in meat was found.

Discussion and Conclusion

In accordance with our findings, Ent2019 administration did not influence the rabbits' growth [3], while another author presented improved weight gain during the application of sage leaves (11.2%) and lantibiotic nisin in rabbits (about 9.4% [6, 32]). Regarding the feed conversion ratio, beneficial impact of Ent2019 and sage extract application in rabbits was noted, and similarly also by us; moreover, increased weight gain and consumption during sage administration was recorded [36]. The improved feed conversion ratio could be explained in terms of more intensive metabolism in the caecum.

Our results concerning the carcass yield are similar to those presented by Celia et al. [8], which showed higher carcass yield during dietary Digestarom® supplementation in weaned rabbits. These facts could be explained by improving nutrient digestibility.

The pH value depends on the balance of muscle energy metabolism (glycogen becomes converted to lactic acid, which leads to the acidification of meat) and is important in maintaining the microbial quality of the meat, because of the bacteriostatic effect of low pH on meats during storage [1]. Although the pH values measured at 24 h after slaughtering were significantly reduced in all experimental groups compared to C (E, S: $P < 0.05$; E + S: $P < 0.001$), they were still in agreement with bibliographic values [41, 42]. The reduced pH_{24} obtained during EntM and sage addition to our rabbits'

Table 3 Physico-chemical composition of rabbits *Longissimus thoracis* and *lumborum* (LTL; means ± SD)

	E	S	E + S	C	*P* value
	Day 21				
Live weight before slaughter (g)	1860.0 ± 69.0^{a}	2273.0 ± 123.0^{b}	1963.0 ± 263.0^{ab}	2255.0 ± 180.0^{b}	0.0116
Bloodless body weight (g)	1780.0 ± 61.0^{a}	2183.0 ± 120.0^{b}	1873.0 ± 255.0^{ab}	2163.0 ± 168.0^{b}	0.0099
Dressed carcass weight (g)	800.0 ± 20.0^{a}	1061.0 ± 68.0^{b}	879.0 ± 141.0^{ab}	1012.0 ± 93.0^{b}	0.0061
Carcass yield (%)	51.58 ± 0.62	53.33 ± 1.01	51.58 ± 1.22	51.80 ± 0.76	0.0581
pH 24 h after killing	5.74 ± 0.08^{a}	5.73 ± 0.09^{a}	5.57 ± 0.08^{a}	5.94 ± 0.12^{b}	0.0012
Water content (g/100 g)	72.26 ± 0.86	72.41 ± 0.29	71.67 ± 0.93	72.53 ± 0.87	0.4467
Protein content (g/100 g)	23.81 ± 0.22^{a}	24.53 ± 0.26^{b}	24.24 ± 0.23^{ab}	23.98 ± 0.19^{a}	0.0039
Fat content (g/100 g)	0.77 ± 0.08^{a}	0.97 ± 0.17^{b}	0.61 ± 0.26^{b}	0.68 ± 0.13^{b}	<0.0001
Electrical conductivity (μS)	1.22 ± 0.48	1.15 ± 0.32	1.24 ± 0.29	0.54 ± 0.21	0.0379
*L** (lightness)	52.36 ± 2.14	52.07 ± 1.63	52.97 ± 3.36	50.49 ± 1.31	0.4473
*a** (redness)	1.38 ± 1.04	1.79 ± 0.44	1.63 ± 1.22	1.38 ± 1.55	0.9431
*b** (yellowness)	8.98 ± 1 .09	8.90 ± 0.34	8.81 ± 0.63	8.38 ± 0.96	0.7308
Water holding capacity (g/100 g)	29.23 ± 2.04	28.69 ± 4.69	29.61 ± 2.72	28.87 ± 1.90	0.9741
Energy value (kJ/100 g)	427.79 ± 2.54^{a}	447.25 ± 10.32^{b}	428.82 ± 6.52^{a}	427.14 ± 7.20^{a}	0.0049
	Day 35				
Live weight before slaughter (g)	2410.0 ± 160.0	2308.0 ± 123.0	2307.0 ± 90.0	2425.0 ± 113.0	0.4032
Bloodless body weight (g)	2320.0 ± 165.0	2183.0 ± 120.0	2228.0 ± 90.0	2268.0 ± 110.0	0.4790
Dressed carcass weight (g)	1169.0 ± 100.0	1061.0 ± 68.0	1099.0 ± 141.0	1147.0 ± 90.3	0.4783
Carcass yield (%)	53.73 ± 1.39	54.92 ± 1.01	54.24 ± 0.83	53.80 ± 0.70	0.3656
pH 24 h after killing	5.88 ± 0.03^{a}	5.82 ± 0.07^{ab}	5.72 ± 0.04^{b}	5.89 ± 0.05^{a}	0.0015
Water content (g/100 g)	72.69 ± 0.62	72.81 ± 0.97	72.90 ± 0.61	71.72 ± 0.87	0.1756
Protein content (g/100 g)	23.89 ± 0.27	24.02 ± 0.14	23.97 ± 0.12	24.21 ± 0.22	0.1826
Fat content (g/100 g)	0.75 ± 0.18	0.93 ± 0.05	0.95 ± 0.28	0.79 ± 0.18	0.3890
Electrical conductivity (μS)	1.39 ± 0.96	0.77 ± 0.23	0.86 ± 0.57	1.75 ± 0.88	0.2317
*L** (lightness)	52.36 ± 2.14	52.07 ± 1.63	52.97 ± 3.36	49.65 ± 5.59	0.5794
*a** (redness)	1.49 ± 1.04	1.79 ± 0.44	1.63 ± 1.22	1.86 ± 1.55	0.9668
*b** (yellowness)	8.98 ± 1.09	8.90 ± 0.34	8.81 ± 0.63	8.38 ± 0.96	0.7308
Water holding capacity (g/100 g)	25.34 ± 3.82	26.92 ± 2.04	27.57 ± 2.84	23.87 ± 1.12	0.2480
Energy value (kJ/100 g)	427.79 ± 2.53^{a}	447.26 ± 10.29^{b}	428.81 ± 6.50^{a}	427.14 ± 7.19^{a}	0.0048

Mean values marked with different letters differ significantly at $P \leq 0.05$

diet are contradictory to the results presented by Meineri et al. [25] who did not noted any differences in the ultimate pH during 10% or 15% chia seed dietary supplementation, and those presented by Cardinali et al. [7] after oregano and rosemary dietary treatments. On the other hand, reduced pH_{48} values in meat samples were measured after oregano, *Eleutherococcus senticosus* and commercial XTRACT application in rabbitries [4] and also when EntM and durancin ED26E/7 were administered to rabbits [14]. Decreased pH value 2 weeks after additive cessation (the end of the experiment) in the E + S group suggests our hypothesis, namely that antimicrobial activity due to lower pH of EntM and sage extract could be noted not only in the gastrointestinal tract of treated rabbits during their application (data not shown), but also in the final product of rabbit meat, increasing the meat's shelf-life and its technological usability.

Meat pH affects many meat properties, including carcass color, water holding capacity, and muscle fat content. The color parameters in particular are strictly related to pH, which influences the muscle texture and the oxidation of heme pigments. The pH_{24} value of meat is negatively correlated with the color parameters L* and b*, which we similarly found but not with the parameter a* [41]. Redness is connected with the degree of iron oxidation in the heme pigment in myoglobin. At high pH levels, oxymyoglobin is rapidly turned into dark red color reduced myoglobin, showing a positive relationship between these parameters [43]; our findings, however, do not confirm this relationship. Moreover, under our conditions, higher values of yellowness were measured, similarly to our previous experiment with phyto-additive application to rabbits [4], and in contrast to Cardinali et al. [7] during oregano and rosemary addition. Increase in yellowness could be connected

Table 4 Fatty acid content in *Longissimus thoracis* and *lumborum* (LTL) 24 h post mortem (% from ΣFA)

	E	S	E + S	C	*P* value
	Day 21				
C12:0 (Lauric a.)	0.067 ± 0.004	0.065 ± 0.003	0.065 ± 0.003	0.060 ± 0.010	0.4004
C14:0 (Miristic a.)	1.311 ± 0.203	1.305 ± 0.038	1.305 ± 0.038	1.330 ± 0.010	0.9841
C16:0 (Palmitic a.)	24.349 ± 0.152	24.473 ± 0.240	24.473 ± 0.240	24.490 ± 0.220	0.7791
C17:0 (Heptadecanoic a.)	0.334 ± 0.034	0.355 ± 0.033	0.355 ± 0.033	0.360 ± 0.052	0.7864
C18:0 (Stearic a.)	10.067 ± 0.004^{a}	10.910 ± 0.295^{b}	10.910 ± 0.295^{b}	11.000 ± 0.220^{b}	0.0003
Σ tested saturated FA	37.439 ± 0.740	37.118 ± 0.745	37.110 ± 0.745	37.240 ± 0.330	0.8852
C18:1n-9c (Oleic a.)	37.856 ± 1.563^{ab}	41.643 ± 1.735^{a}	38.281 ± 4.437^{b}	39.530 ± 1.750^{ab}	0.0039
C18:1 11c/15t (Vaccenic a.)	4.715 ± 0.049^{ab}	4.695 ± 0.064^{ab}	4.846 ± 0.172^{a}	4.610 ± 0.090^{b}	0.0515
C20:1 (Eicosenoic a.)	0.551 ± 0.029^{ab}	0.602 ± 0.036^{ab}	0.664 ± 0.108^{a}	0.530 ± 0.020^{b}	0.0342
Σ tested monounsaturated FA	43.122 ± 0.943^{a}	46.940 ± 1.364^{b}	43.791 ± 0.408^{a}	44.670 ± 1.410^{a}	< 0.0001
C18:2n-6 (Linoleic a.)	9.760 ± 1.420	11.080 ± 0.910	9.860 ± 0.430	10.840 ± 0.780	0.1696
C18:2 9c/11t (CLA)	0.139 ± 0.004	0.146 ± 0.006	0.134 ± 0.013	0.130 ± 0.010	0.1013
C18:3n-3 (α-Linolenic a.)	0.251 ± 0.008	0.242 ± 0.012	0.253 ± 0.011	0.250 ± 0.030	0.8222
C20:4n-6 (Arachidonic a.)	1.927 ± 0.203^{ab}	2.059 ± 0.020^{a}	1.899 ± 0.072^{ab}	1.640 ± 0.260^{b}	0.0281
C20:5 n-3 (Eicosapentaenoic a.)	0.111 ± 0.003^{ab}	0.122 ± 0.009^{a}	0.110 ± 0.015^{ab}	0.100 ± 0.010^{b}	0.0663
C22:5 n-3 (Docosapentaenic a.)	0.130 ± 0.005	0.139 ± 0.008	0.133 ± 0.002	0.140 ± 0.010	0.1824
C22:6 n-3 (Docosahexaenic a.)	0.033 ± 0.004	0.037 ± 0.006	0.032 ± 0.004	0.040 ± 0.000	0.0615
Σ tested polyunsaturated FA	12.351 ± 0.125^{a}	13.825 ± 0.743^{a}	12.421 ± 0.707^{b}	13.140 ± 0.290^{ab}	0.0067
other not tested FA	7.088 ± 0.086 ^{a}	2.117 ± 0.054 ^{b}	6.678 ± 0.103 ^{c}	4.950 ± 0.087 ^{d}	< 0.0001
PUFA n6	11.687 ± 1.217	13.139 ± 0.710	11.759 ± 0.380	12.480 ± 0.570	0.0762
PUFA n3	0.525 ± 0.005	0.540 ± 0.006	0.528 ± 0.004	0.530 ± 0.015	0.1363
n6/n3	22.260 ± 0.002^{a}	24.330 ± 0.003^{b}	22.270 ± 0.004^{c}	23.550 ± 0.004^{d}	< 0.0001
Fat content (g/100 g)	0.77 ± 0.08^{a}	0.97 ± 0.17^{b}	0.61 ± 0.26^{b}	0.68 ± 0.13^{b}	< 0.0001
Cholesterol (g/100 g)	0.303 ± 0.017	0.307 ± 0.021	0.335 ± 0.045	0.300 ± 0.050	0.5233

Mean values marked with different letters differ significantly at $P \leq 0.05$

with free radicals, produced by lipid oxidation during meat storage and/or manipulation, which can oxidize haem pigments, causing discoloration of meat and meat products [44]. The meat lightness is negatively correlated with pH value, i.e., the lower the pH, the clearer the lightness [45]; our results also supported this finding. Chrastinová et al. [14] and Pogány Simonová et al. [46] also demonstrated that the physico-chemical parameters and nutritional value of rabbit meat were not negatively influenced during EntM, durancin ED26E/7 and nisin application; these were pilot studies regarding bacteriocin effects on rabbit meat quality. Our results are novel, moreover, as the effect of EntM on meat color parameters has not been tested in rabbits up to now.

Decrease in water content in all experimental groups, mainly in E + S, could be explained by the high adstringence effect of sage on mucus due to its thujone content; this fact was noted also in serum parameter testing (data not shown), which indicated losses of water in meat as well. Meat pH decreases with post-mortem metabolism progress, and this reduces net protein charges because the muscle proteins are closer to the isolelectric point which leads to protein denaturation, lower hydration level, and diminished water holding capacity (WHC [45]). Other water losses in meat continously promote pH reduction and negatively influenced the WHC. Generally, our results on water content agree with other researchers; lower water content levels were noted in all experimental groups, mainly in E + S compared with C and found to be positively correlated with reduced pH. On the other hand, the WHC decreased only in S group, in contrast to other findings. Electrical conductivity (EC) is an electrical characteristic of muscle/meat which changes with time due to post-mortem glycolysis and it is an indicator of membrane integrity, permeability, and ion transport [47]. A necessary effect of water losses is increased EC; this was also comfirmed by us during EntM and sage administration to rabbits, compared to untreated animals. Our results are, however, contrary to those presented by Chrastinová et al. [14], applying EntM and durancin ED26E/7 to rabbits. There is also positive correlation between WHC and intramuscular fat content [48]. The lipid content of rabbit meat mostly depends on the portion considered and especially on feeding—it ranged from 0.6 to 14.4 g/100 g (6.8 g/100 g is the average [1]), whereby the loin (*M. longissimus dorsi*) was

Table 5 The content of essential amino acids in *Longissimus thoracis* and *lumborum* (LTL) in rabbits (g/100 g)

	E	S	E + S	C	*P* value
	Day 21				
Total protein (g/100 g)	23.81 ± 0.22[a]	24.53 ± 0.26[b]	24.24 ± 0.23[ab]	23.98 ± 0.19[a]	0.0039
Arginine	1.362 ± 0.088	1.222 ± 0.095	1.298 ± 0.032	1.400 ± 0.150	0.1172
Cysteine	0.295 ± 0.023	0.284 ± 0.018	0.271 ± 0.020	0.300 ± 0.030	0.3412
Phenylalanine	0.887 ± 0.054	0.806 ± 0.056	0.839 ± 0.027	0.910 ± 0.090	0.1228
Histidine	0.951 ± 0.080	0.865 ± 0.080	0.863 ± 0.080	0.990 ± 0.100	0.1395
Isoleucine	0.830 ± 0.055	0.793 ± 0.057	0.794 ± 0.050	0.850 ± 0.090	0.5447
Leucine	1.705 ± 0.112	1.540 ± 0.109	1.615 ± 0.050	1.750 ± 0.180	0.1221
Lysine	1.823 ± 0.118	1.638 ± 0.126	1.731 ± 0.048	1.870 ± 0.200	0.1246
Methionine	0.674 ± 0.041	0.628 ± 0.043	0.628 ± 0.037	0.700 ± 0.070	0.1566
Threonine	0.962 ± 0.080	0.881 ± 0.047	0.914 ± 0.024	0.970 ± 0.090	0.2354
Valin	0.954 ± 0.080	0.883 ± 0.038	0.907 ± 0.025	0.970 ± 0.070	0.1744
Essential amino acids	10.440 ± 0.764	9.485 ± 0.663	9.858 ± 0.331	10.700 ± 1.230	0.1944

Mean values marked with different letters differ significantly at $P \leq 0.05$

the leanest portion (1.8 g/100 g [5]). Although fat content seemed to be higher in meat from the E and S groups compared with that from C and E + S, in our case lower fat content was measured than that presented by the authors cited above. Comparing all meat samples, the highest fat and the highest protein contents were measured in meat samples from rabbits receiving sage extract, similarly to Meineri et al. [25] after 10% and 15% chia seed supplementation. Increased fat concentration was also recorded during phyto-additives and gallidermin application in broiler rabbits in comparison with control data [4, 49]. In this experiment. the highest protein content so far was achieved among all probiotic strains and their bacteriocins isolated by our team, as well as the commercial nisin and gallidermin and phyto-additives tested by us in rabbits [3, 4, 14, 36, 46]. There is a close correlation between protein and lipid contents and the energy value of meat, confirmed in this study and also in our previous studies with experimental application of oregano, sage, and *Eleutherococcus senticosus* extracts in rabbits [4].

The meat nutritional value is usually confirmed by its high content of AAs, FAs, minerals, and vitamins. The percentage of SFA, MUFA, and PUFA was found in a similar range as it was measured by Chrastinová et al. [14] after EntM and Durancin ED26E/7 administration to rabbits. In general, the MUFAs were measured in higher and the PUFAs were noted in lower concentrations than it was observed after sage and thyme supplementation to rabbits [11, 12]. As expected, the FA composition of the LTL was influenced by dietary inclusion with sage in favor of unsaturated FAs, similarly to Peiretti and Meineri [11]; the concentrations of MUFAs decreased, while PUFAs' levels increased after sage administration. Both the EntM and sage extract increased the level of arachidonic acid, similarly to other findings after enterocin and chia seed dietary inclusion to rabbits [11, 14]. Among the PUFAs, linoleic and linolenic are essential fatty acids, because animal organisms are unable to synthetize them; both of them were elevated (even numerically, not significantly) using sage separately (for linoleic acid) and in combination with EntM (for

Table 6 The content of minerals in *Longissimus thoracis* and *lumborum* (LTL) in rabbits (g/100 g)

	E	S	E + S	C	*P* value
	Day 21				
Calcium (mg/100 g)	5.60 ± 0.01	6.50 ± 0.02	6.50 ± 0.01	5.50 ± 0.01	0.5107
Phosphorus (mg/100 g)	237.00 ± 0.05	239.30 ± 0.09	230.10 ± 0.09	225.20 ± 0.07	0.0923
Magnesium (mg/100 g)	21.30 ± 0.006[a]	22.00 ± 0.008[b]	21.10 ± 0.005[a]	21.40 ± 0.005[a]	0.0031
Natrium (mg/100 g)	29.80 ± 0.04	27.40 ± 0.05	34.00 ± 0.02	27.60 ± 0.02	0.0619
Potassium (mg/100 g)	361.80 ± 0.22[ab]	382.20 ± 0.17[a]	381.90 ± 0.07[a]	339.40 ± 0.06[b]	0.0038
Iron (mg/100 g)	0.396 ± 0.827	0.481 ± 0.992	0.481 ± 0.885	0.468 ± 1.330	0.6146
Manganese (mg/100 g)	0.058 ± 0.109	0.069 ± 0.205	0.063 ± 0.062	0.058 ± 0.220	0.7150
Zinc (mg/100 g)	1.138 ± 3.167	1.344 ± 8.466	1.624 ± 3.944	1.890 ± 8.229	0.4084
Copper (mg/100 g)	0.203 ± 0.198	0.177 ± 0.109	0.181 ± 0.142	0.295 ± 1.808	0.2871

Mean values marked with different letters differ significantly at $P \leq 0.05$

linolenic acid). Linoleic acid is the precursor of *n*-6 PUFAs, while linolenic acid of the *n*-3 PUFAs, especially of EPA and docosahexaenic (DHA) acids, which are the most bioactive FAs. The majority of studies showed the great ability of rabbits to synthesize long chain PUFA (EPA and DHA) from dietary precursors, increase the *n*-3 PUFA content of their meat, and reduce its *n*-6/*n*-3 ratio [5]. Rabbit meat has a very low amount of EPA and DHA content [50]; therefore, increasing the EPA concentration by sage addition is an interesting goal to improve nutritional quality of rabbit meat. The total amount of *n*-6 and *n*-3 PUFAs was lower than it was presented by other researchers [5, 11, 12]. The *n*-6/*n*-3 ratio is generally very high in rabbit meat, beacuse of high content of linoleic acid [1]. In the present study, the ratio of *n*-6 to *n*-3 FAs reached 22.26–24.33 for LTL, which is much higher than it was described by the authors mentioned before. In contrast to Peiretti and Meineri [11], who described decrease in *n*-6/*n*-3 ratio during chia seed administration to rabbits, no effect of sage addition on this parameter was observed by us.

No significant differences from control data were observed in the essential AA composition of LTL muscles through the additives application. In general, tested AA concentrations varied in the range from 9.49 to 10.70 g/100 g and were in agreement with those presented by Chrastinová et al. [14], but they were lower compared to phyto-additive application to rabbits [4]. The concentrations of tested essential AAs were similar to those presented by Dalle Zotte [1], except histidine and arginine, which showed lower values than it was found by us. Moreover, the all tested AAs were found in lower levels than in control animals, as was described also by Chrastinová et al. [14] through enterocins addition to rabbits. In contrast to these results, an increase of threonine, leucine, and phenylalanine levels was noted after Ent4231 administration and higher threonine, leucine, phenylalanine, and histidine concentrations were found through phyto-additive consumption in rabbits [3, 4]. The comparison of our results with literature data is rather difficult, because there are only some available studies about rabbit meat AA composition, presenting the influence of weaning age, different rabbit breed and their crosses and the probiotic Bioplus 2B® preparation on the AA content of rabbit meat [20–22]. Although the essential AAs were not influenced by EntM and sage administration, outgoing from higher levels of protein content in rabbits receiving sage extract separately and in combination with EntM, we hypothesize that these additives could improve the non-essential AAs levels, which were not measured by us.

There is a great variaty in macrominerals and trace element content of rabbit meat between different studies. Outgoing from increased levels of tested minerals in experimental groups, improved jejunal morphometry, and cecal enzymatic activity through EntM and sage administration to rabbits (data not shown), we hypothesise better mineral inclusion to rabbit meat due to enlargement of luminal and absorption surface and increased mineral absorption improved by short chain fatty acids.

The calcium and phosphorus contents framed within those reported in the literature by Dalle Zotte and Szendrő [5] and Nistor et al. [18], while potassium and sodium level was below the range presented by these authors. Calcium is an essential element in bone mineralization. Higher calcium levels were noted in groups S and S + E, which could be explained by the positive effects of phytoestrogenic compounds in sage, having structural similarities to estrogen conformation and binding capabilities to estrogen receptors, which may therefore promote calcium absorption through an estrogen receptor pathway within intestinal cells. Elkomy and Elsaid [51] also presented that feeding sage, rosemary, and thyme were able to restore decreased levels of serum Ca and P to normal values and modulate bone loss in ovariectomized rats. Phosphorus is the second most abundant mineral in meats and rabbit meat is characterized by its high content. Similarly to calcium, its improved inclusion to meat could be explained by phytoestrogenic compounds in sage, and in the case of EntM, we hypothesize increased transcellular phosphorus uptake transporter expression at the cell. Rabbit meat is highly recommended for its high magnesium and potassium content; these mineral contents were significantly higher through EntM and sage administration. Rabbit meat is richer in potassium, "electrolyte" important to both cellular and electrical function, than other types of meat [1]; high potassium and low sodium level may make rabbit meat particularly recommended for hypertension diet. Despite the fact that potassium content of rabbit meat significantly increased through the sage administration compared to C, these values were still lower than it was presented by Dalle Zotte and Szendrő [5]. On the other hand, we found reduced potassium values after CCM7420 strain application [19].

Although meat represents the main dietary source of highly available iron, it is important to know the respective amounts of heme and non-heme iron; the latter is less readily absorbed than the former iron form. Furthermore, cooking processes can transform heme into non-heme iron, and then change total ironavailability. Iron was measured in lower values in comparison with other studies [5, 15, 19]. Hernández and Gondret [50] reviewed that rabbit meat has the lowest zinc concentration among different types of meats, and that copper concentration is quite similar to the other species analyzed. While zinc and copper concentrations were decreased through EntM and sage application, their values were still higher than it was presented by Hernández and Gondret [50] and compared to data achieved after CCM7420 strain application [19].

In conclusion, it seems that EntM and sage extract could be involved in rabbit diet without any adverse effects on the carcass characteristics. Dietary supplementation, mainly with sage extract, is effective in improving the carcass yield, protein content, and energy value of rabbit meat. These findings are also supported by good health status of rabbits and

enhanced the protein content and energy value of rabbit meat. These findings are also supported by good health status of rabbits. The sage extract dietary inclusion was also effective in improving of the PUFA content, mainly the levels of arachidonic and eicosapentaneic acids of the rabbit meat. The mineral profile of rabbit meat was also positively affected with a particularly significant elevation of its potassium and magnesium content and numerical increase of phosphorus, iron and manganese concentrations. The AA composition was not influenced by sage addition to rabbits diet. The EntM administration did not significantly changed the FA, AA and mineral concentration of rabbit meat; only numerical increase of the phosphorus, natrium, potassium, and manganese concentrations in meat was found. We conclude that diet supplementation with sage extract could enhance the nutritional quality of rabbit meat, with the focus on PUFA and minerals. Our results are novel, moreover, as the effect of EntM on meat color parameters, on FA and AA composition has not been tested in rabbits previously.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval The experiment was performed in cooperation with our colleagues at the National Agricultural and Food Centre (NAFC, Nitra, Slovakia). Institutional and national guidelines for the care and use of animals were followed appropriately, and all experimental procedures were approved by the Slovak State Veterinary and Food Administration and Ethics Committees of both (permission code: SK CH 17016 and SK U 18016). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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