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Featured Application: Postbiotic MLS EM41/3 application study indicates its promising use in animal husbandry. It supports also the fact that its administration in horses with beneficial effect was also assessed by our team. Results will be summarized in further.

Abstract: Nowadays, animal breeders look for effective innovations to care animal health. Use of probiotics, postbiotics, parabiotics and the other beneficial natural substances are dominated. Mundticin-like substance EM 41/3 is thermo-stable bacteriocin (postbiotic) with a broad antimicrobial spectrum. In this study its effect in broiler rabbits was assessed. Rabbits represent food-derived animal and they are also suitable animal model. The parameters tested were analyzed by validated methods. Administration of MLS EM 41/3 produced by non-autochthonous strain *Enterococcus mundtii* EM 41/3 lead to significant increase of parameter immunity-phagocytic activity, while it did not influence microbiota composition. It also did not influence blood biochemistry and no oxidative stress was noted. Moreover, higher growth parameters were noted and also stimulated hydrolytic activity. Finally, no negative influence on rabbit meat quality was noted. Postbiotic MLS EM 41/3 administration in feeding strategy seems to be contributing fact for rabbit health status maintaining.

Keywords: bacteriocin; broiler rabbits; postbiotic effect; health status

1. Introduction

Nowadays, continually have been searched various innovative ways to maintain healthy status of animals, food-derived animals involving. Among food-derived animals also belong broiler rabbits because of their easily digestible meat [1] which could be recommended for people e.g., in the period after illness convalescence. Rabbits can convert an approximately 20% of the protein they eat into edible meat which is higher amount that e.g., in pig meat (16-18%) [2]. Among innovations, use of probiotics, postbiotics, parabiotics and the other beneficial natural substances are dominated [3]. Recently, more frequently postbiotics have been aimed for this purpose. Postbiotic components are diverse and outperform live probiotics in terms of technology, safety, cost due to their sufficient absorption, metabolism and organismal distribution [4]. This term has been officially established by the ISAPP (The international Scientific Association of Probiotics and Prebiotics consensus statement on the definition and scope of postbiotics) [5]. There has been even reported future perspectives for postbiotics in a very wide ways, health care involving.

Mundticin-like substance EM 41/3 is thermo-stable bacteriocin previously reported by Focková et al. [6]. It is produced by horse strain *Enterococcus mundtii* EM 41/3 (isolated from Slovak breed

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Norik from Muráň) [6]. This substance showed the broad antimicrobial spectrum. It is predominantly aimed for horse health status keeping [6]. However, in this study its effect in broiler rabbits has been assessed because rabbits besides they are food-derived animal category are also suitable model animals. The reasons are, e.g., the small body size, short generation interval, rapid growth rate, high productive capacity, and their meat as formerly mentioned [1,7]. Previously, some other promising results with bacteriocins (postbiotics) or their producer strains were presented by our team [7–10]. E.g. Enterocin M and Durancin ED26E/7 application lead to coliforms decrease *($p<0.001$)* in faeces of rabbits, and pseudomonads as well *($p<0.05$).* Both postbiotics (bacteriocins) stimulated caecal enzymatic activity [7–10]. In case of Ent M also higher daily weight gains were noted, stimulation of PA ($p<0.01$, $p<0.001$) not only in case of Ent M but also in case of its producer strain EF41=CCM8558 [9]. In the presented study microbiota were checked, phagocytic activity (PA), GPx to assess/eliminate oxidative stress, growth parameters, biochemistry in blood serum, jejunal morphology, organic acids in caecal chyme, faecal enzymatic activity, and meat quality. These aims have been conducted to secure rabbits health as food-derived animals and their meat as functional food.

2. Materials and Methods

2.1. Preparation for Application Mundticin-like Substance EM 41/3

Mundticin-like substance EM 41/3 is bacteriocin produced by the strain *Enterococcus mundtii* EM 41/**3** [6]. This bacteriocin-producing strain was isolated from faeces of Slovak horses breed Norik from Muráň. To apply it in broiler rabbits , the strain was inculated in MRS broth (Merck, Darmstadt, Germany, pH 6.9, 0.1% inculum). Based on previous testing to find its bacteriocin production, it was cultivated in the late log phase in incubateor at 37 ˚C. Broth culture was then centrifuged (10 000 x *g*) for half hour. Supernatant obtained (pH 5.5) was treated with EDTA III (Sigma, Germany) and it was exposed to the temperature 80 ˚C for 10 min to inactivate other organic components than bacteriocin. Then supernatant was precipitated with ammonium sulphate at 4 ˚C for 18 h (40% saturation). Precipitate was re-suspended in minimal volume of phosphate buffer (10 mM, pH6.5). Inhibitory activity was tested by the agar spot method [11] against two indicator strains; *Enterococcus avium* EA5 (faecal piglets strain, from our laboratory) and *Listeria monocytogenes* LMP 7223 (State Veterinary Institute in Olomouc, Czech Republic). Inhibitory activity of this precipitate reached 25 600 AU/mL and/or 102 400 AU/mL.

2.2. Experimental Design and Sampling

A total 48 rabbits (meat lines M91 and P91) after weaning (in age 35 days), both sexes (equal male-to-female ratio per treatment) were divided into the experimental group (EG) and the control group (CG), 24 animals in each. The average body weight of rabbits at the start of experiment was 1 041 g (EG)-1078 g (CG). The rabbits were placed in the menagerie of the National Agricultural and Food Centre (NAFC) in Nitra-Lužianky. It is workplace with which we have co-operated almost for 20 years. The guidelines stated in the Guide for the care and use of Laboratory Animals approved by the Slovak Veterinary and Food Administration and Ethical Commissions of both institutions (permission code:SK CH 17016 and SK U 18016) were accepted for care and experimental procedures. The animals were fed a commercial diet for growing rabbits (SIGI Trade, Dvory nad Žitavou, Slovakia) with the following nutritional values: dry matter 896.79 g/Kg, crude fiber 140.29 g/Kg, fat 23.84 g/Kg, N-substances 172.83 g/Kg, ash 93.68 g/Kg, organic matter 803.11 g/Kg, starch 167.66 g/Kg. The minerals in the diet were supplemented in amount such as magnesium- 3.27 g/Kg, sodium-1.25 g/Kg, kalium-13.22 g/Kg, iron- 626.97 mg/Kg, and zinc-173.02 mg/Kg. Energy value of the diet was 11.22 MJ/Kg. Broiler rabbits were kept in standard cages (type D-KV-72; 0.61 m x 0.34 cm x 0.33 m; Kovobel company Domažlice, Czech Republic), two animals per cage. A cycle of 16 h light and 8 h dark was applied throughout the experiment. The temperature and humidity in building with rabbits were recorded continously by a digital thermograph positioned at the same level as the cages. The heating and ventilation systems allowed the menagerie air temperature maintained within 16 ± 4 ˚C

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and the relation humidity to about 70 ± 5 % throughout experiment as previously reported Pogány Simonová et al. [12]. Rabbits has had attitude to drinking water *ad libitum.*

Animals were administrated with Mundticin-like substance (MLS) EM 41/3 in drinking water, 50 µL for animal per day during 21 days. Experiment lasted for 42 days. Sampling was performed at day 0/1 (*n=10,* before MLS application), at day 21 (*n=5*, 3 weeks of MLS application), and at day 42 (*n=5*, 3 weeks of MLS cessation). Mixture of faeces were analyzed; at days 21 and 42 also caecum (*n=4*) and appendix (*n=4*) were sampled after rabbits slaughtering as previously described by Pogány Simonová et al. [12], one rabbit/one replicate, selected based on daily weight measurement to ensure similar weight of animals. Faecal, caecal samples were also sampled to test hydrolytic activity.

Musculus longissimus thoracis and lumborum (MLTL) was separated by removing skin, connective tissue, chilled and stored at 4 ˚C for 24 h until analysis. Rabbits were also regularly weighted.

Rabbits blood (*vena auricularis*) was sampled in Eppendorf tubes (with and without heparin according to parameter analyzed) at day 0/1, at days 21 and 42.

2.3. Microbiota Analyses

Microbiota were checked in faeces, caecum and appendix. Fo their enumeration the standard dilution microbiological method was used (ISO, International Organization for Standardization) with the selective media (ISO). Faecal samples as well as samples of caecum and appendix (1 g) were mixed with Ringer solution (ratio 1:9, Merck, Darmstadt, Germany, pH=7.0) and treated using Stomacher-Masticator (Spain). The appropriate dilutions were spread on M-Enterococcus agar (Becton and Dickinson, Difco, Detroit, MI, USA) for the total count of enterococci. Mannitol salt agar (MSA, Difco) was used for enumeration of staphylococci. To enumerate coliforms, MacConkey agar (Oxoid, Basingstoke, the United Kingdom) was used and amylolytic streptococci were counted on M17 agar (Difco) enriched with starch. The agar plates were incubated according to bacteria growth expected at 37 ˚C for 24-48 h. Bacterial counts were expressed in colony forming unit per gram (CFU/g) log 10 ± SD.

After re-isolation of colonies from different agar media, colonies were treated with MLS EM 41/3 using agar spot test [11] and inhibitory activity was expressed in arbitrary units per milliliter (AU/mL).

2.4. Phagocytic Activity Analysis, Glutathione-Peroxidase-GPx Evaluation, and Biochemistry in Blood Serum

For phagocytic activity (PA) blood (*n=8)* from *vena auricularis* was sampled into Eppendorf tubes with micro-spheric hydrophilic (MSH) particles and heparin to test phagocytic activity (PA) [13]. Sampling was performed at days 0/1, 21, and 42. The volume 50 µL of MSH particle suspension (ARTIM, Prague, Czech Republic) was mixed with 100 µL of blood in an Eppendorf tubes and incubated at 37 ˚C for 1 h. Blood smeares were prepared and stained with May-Gruenwald and Giemsa-Romanowski. To validate PA, the direct microscopic counting procedure was performed with calculation of the number of white cells containing at least three engulfed particles per 100 white cells (monocyte/granulocytes). PA was expressed in percentage (%). Also index of phagocytic activity was involved in analysis (IPA).

GPx-glutathione-peroxidase activity (*n=8*) was determined by the colorimetric method (Spectrophotometer UV-2550 Shimadzu Japan) using the commercial kit Randox RS504 (Randox Laboratory) after blood sampling in the tube with heparin.

For biochemistry, blood samples were taken into an Eppendorf tubes. They were centrifuged (3 000 *x g,* 30 min) and delivered in SK-Lab company Lučenec (Slovakia). For analyzing, validated methods were used. The following parameters were analyzed: total proteins (TP in g/L), albumine (g/L) and kreatinine (µmol/L), alanine aminotransferase (ALT in µkat/L), aspartate transferase (AST in µkat/L), alkalic phosphatase (ALP in µkat/L), glucose (mmol/L), cholesterol (mmol/L), triglycerides (mmol/L), sodium (Na), kalium (K), chlorides (CL) calcium (Ca), phosphorus (P), and magnesium (Mg) in mmol/L. Reference values for rabbits are summarized in Table 4.

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2.5. Hydrolytic Activity, Jejunal Morphometry Growth Performance, Organic Acids in Chyme, Quality of Meat, Statistical Assessment

Hydrolytic activities, amylolytic, cellulolytic, xylanolytic, pectinolytic and inulolytic (expressed in μmol/g/DM/min meaning in mikromol per gram of dry matter per minute) were processed as previously described Lauková et al. [14]. The enzymes were extracted using the procedure of Huhtanen and Khali [15] and they were measured according to procedure by Miltko et al. [16].

Body weight (BW) was measured every week during the experiment; average daily weight gain (ADWG) and feed conversion ratio (FCR) were calculated mathematically.

MLTL (100 g) were stored for 24 h *post mortem* at 4 °C. The following parameters were analyzed: total water content (g/100g), total proteins (g/100 g), total fat, water holding capacity, pH 24, energy value (kJ/100 g) using validated methods (analyzator INFRATEC 1265) in co-operation with colleagues in Nitra-Lužianky as well as method according to Ouhayoun [17] by gas chromatography (for organic acids). The pH values were measured 24 h *post mortem* with a Radelkis OP-109 pH meter (Jenway, England). Morphometry testing was performed as previously described Žitňan et al. [18].

Treatment effect regarding the tested parameters was statistically analyzed using one-way analysis of variance (ANOVA) with Tukey post hoc test. Data are expressed as means and standard deviation SD of the mean. Different superscript indicated significant difference $p<0.05$. Statistical analyses were performed by the using GraphPad Prism version 6.0 (San Diego, CA, USA).

3. Results

3.1. Microbiota Evaluation

The total counts of enterococci in rabbits faeces at day 21 reached the value 3.39 ± 1.45 CFU/g (log 10) in average (Table 1). At day 21 their counts were signigficantly higher in EG comparing to day 0/1 ($^{ab}p<0.001$) but also comparing EG to CG at day 21 ($^{bc}p<0.001$). Also at day 42 were found higher counts of the total enterococci in EG comparing to CG; however, with mathematical difference (1.19 cycle) and also comparing to day 0/1 (mathematical difference 1.35 cycle). It looks that enterococci were not reduced by MLS EM 41/3. Similarly, the total counts of other checked bacteria in faeces of broiler rabbits were not influenced (LAB, staphylococci, amylolytic streptococci and coliforms, Table 1).

Table 1. Faecal bacterial counts in rabbits after MLS EM 41/3 application and its cessation.

	Day 0/1 (*n=10*)	Day 21 (*n=5*)-EG	Day 21 *(n=5)-CG*	Day 42 *(n=5)*-EG	Day 42 *(n=5)-CG*
Enterococci	3.39 ± 1.45^{a}	5.81 ± 0.22^{b}	4.40 ± 0.79^{c}	4.74 ± 0.47	3.39 ± 0.48
Lactic acid bacteria	3.79 ± 1.23	5.67 ± 0.22	4.94 ± 0.75	4.44 ± 0.82	3.25 ± 0.40
Staphylococci	3.98 ± 0.50	4.28 ± 0.34	3.91 ± 0.33	4.25 ± 0.28	4.43 ± 0.57^{a}
Amyl. streptococci	5.71 ± 0.21	5.76 ± 0481	5.24 ± 0.61	5.99 ± 0.37	5.89 ± 0.24
Coliform bacteria	2.28 ± 1.72	5.37 ± 0.73	4.83 ± 1.16	4.18 ± 1.27	3.85 ± 0.64

Average value of bacteria in CFU/g (log 10) ± SD; Enterococci, $^{ab}p< 0.001$; $^{bc}p<0.001$; Amylolytic streptococci, Day 0/1, sampling before application, EG/21, experimental group at day 21, CG/21, control group at day 21, EG/42, experimental group at day 42, CG/42, control group at day 42;.

Enterococcal counts in caecum were lower than in faeces. At days 21 and 42 they reached up to 1.5 CFU/g (log 10) (Table 2). At day 21, LAB in caecum of EG were mathematically reduced (difference 0.67 cycle) comparing to CG. Staphylococcal count in caecum and faeces was almost the same in EG at day 21 (Tables 1 and 2). However, at day 21 staphylococci were slightly reduced in caecum (difference 0.11 cycle) and at day 42 even significant difference was found in EG comparing to CG ($^{ab}p<0.05$). Amylolytic streptococci in caecum were almost 1.0 log cycle lower than in faeces (Table 2)

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and they were slightly higher in EG than in CG (difference 0.19 respectively 0.22 cycle) at day 21 and 42 as well.

Enterococci in appendix reached up to 2.54 CFU/g (log 10) and at day 42 up to 1.0 CFU/g; they were higher in EG than in CG. Also LAB were higher in EG than in CG at day 21, later (at day 42) they decreased up to almost 1.0 CFU/g. Coliforms and amylolytic streptococci reached counts almost similar as counted in caecum at day 21 and 42 and they were not reduced.

Picked up bacterial colonies re-isolated from faeces, caecum and appendix were mostly susceptible to MLS EM 41/3 (50%).

Table 2. Bacterial counts in caecum and appendix in rabbits after MLS EM 41/3 application and its cessation.

	Day 21 (*n=4*)-EG	Day 21 *(n=4)-CG*	Day 42 *(n=4)*-EG	Day 42 *(n=4)*-*CG*
Caecum				
Enterococci	0.93 ± 0.05	0.93 ± 0.05^{c}	1.43 ± 1.05	0.09 ± 0.0
Lactic acid bacteria	1.82 ±1.41	2.55 ± 1.93	1.05 ± 0.17	0.95 ± 0.06
Staphylococci	3.96 ± 0.29	4.07 ± 0.51	2.48 ± 1.72^{b}	4.09 ± 0.30^{a}
Amylolytic streptococci	4.15 ± 0.21	3.96 ± 0.39	4.43 ± 0.19	4.21 ± 0.16
Coliform bacteria	2.42 ± 1.25	1.32 ± 0.33	3.54 ± 1.04	2.42 ± 1.25
Appendix	Day 21 (*n=4*)-EG	Day 21 *(n=4)-CG*	Day 42 *(n=4)*-EG	Day 42 *(n=4)*-*CG*
Enterococci	2.54 ± 1.07	0.93 ± 0.05	0.90 ± 0.00	0.90 ± 0.0
Lactic acid bacteria	3.30 ± 1.63	2.55 ± 1.93	0.90 ± 0.00	0.95 ± 0.06
Staphylococci	3.78 ± 0.24	4.07 ± 0.51	3.59 ± 0.23	4.09 ± 0.30
Amylolytic streptococci	4.47 ± 0.42	3.96 ± 0.39	4.66 ± 0.38	4.21 ± 0.16
Coliform bacteria	3.97 ± 0.17	1.32 ± 0.33	4.95 ± 0.11	2.42 ± 1.25

Average value of bacteria in CFU/g (log 10) ± SD; Day 0/1, sampling before application, EG/21, experimental group at day 21, CG/21, control group at day 21, EG/42, experimental group at day 42, CG/42, control group at day 42;.

3.2. Phagocytic Activity, GPx and Biochemistry

PA at day 0/1 was 57.13 ± 3.14 % (Table 3). At day 21, PA was increased in EG comparing to day 0/1 but also to CG ($^{ab}p<0.05$, $^{bb}p<0.05$). PA in EG was also increased at day 0/1 comparing to day 42 ($^{bc}p<0.05$). Increase in PA was also noted in CG at day 21 comparing to day 0/1 ($^{ab}p<0.05$) and comparing day 0/1 to day 42 as well ($^{ac}p<0.05$). At days 21 and 42 was PA almost at the same level in CG (Table 3). IPA values were not influenced.

The values of GPx were increased in both groups (EG, CG) at day 21 (Table 3) comparing to day 0/1; however, in CG the value was higher than in EG (no oxidative strain stimulation). At day 42, GPx values decreased; in EG even on the almost initial level (Table 3). And still lower in EG than in CG.

Table 3. The values of phagocytic activity (=), IPA, and GPx (U/gHb).

n=8	Day 0/1	Day 21	Day 42
EG/PA	57.13 ± 3.14^{a}	64.38 ± 1.30^{b}	60.13 ± 0.99^{c}
CG/PA	57.13 ± 3.14^{a}	60.13 ± 0.99^{b}	60.38 ± 0.92^{c}
EG/IPA	3.55 ± 0.38	3.88 ± 0.05	3.40 ± 0.20
CG/IPA	3.55 ± 0.38	3.86 ± 0.05	3.88 ± 0.05

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EG/GPx	151.98 ± 27.32	185.98 ± 37.60	153.05 ± 29.14
CG/GPx	151.98 ± 27.32	190.86 ±28.98	171.49 ± 27.66

EG-experimental group, CG-control group, GPx-glutathione-peroxidase, ± SD;Day 21, EG: Day 0/1, EG: CG ($^{ab}p<0.05$, $^{bb}p<0.05$). EG day21: day 42 ($^{bc}p<0.05$). CGt day 21: day 0/1 (^{ab} $p<0.05$), day 0/1: day 42 ($^{ac}p<0.05$). GPx, NS, only mathe-matical increase.

The total protein values were lower than ranging limit values; however, they were increased in both groups at day 21 comparing to day 0/1 and they were stable in EG also at day 42 (Table 4). The same situation was noted with albumine status and measured values were in ranging limit. In case of kreatinine, significant increase was noted (Table 4) in framework of reference ranging limit. Bilirubin values in all groups reached less than 0.3 μkat/L and were under limit value. Glucose values were in ranging limit and significantly decreased at days 21 and 42 comparing to day 0/1 ($p <0.001$), still in top limit level (Table 4). Cholesterol was in ranging limit and significantly decreased ($p <0.01$). Triglycerides were slightly higher at day 0/1 but in ranging limit. At days 21 and 42 they were slightly decreased to be in limiting range (Table 4). ALP was higher at day 0/1 Then ALP values were decreased in to value of ranging limit. AST value were low and values of ALT were also under limit (Table 4). Na and K values were balanced, Ca value was in limit range as well as Mg and P. Chlorides were also in optimal range and slightly increased in all groups.

Table 4. Biochemistry.

n=8	Day 0/1	EG/21	CG/21	EG/42	CG/42
Total protein (g/L)	42.65 ± 3.06	47.41 ± 7.39	49.33 ± 6.36	47.03 ± 3.29	48.18 ± 5.30
Albumine (g/L)	31.69 ± 2.22	33.61 ± 5.60	34.35 ± 4.39	33.44 ± 2421	32.83 ± 2.46
Kreatinine (μmol/L)	27.97 ± 3.21^{a}	43.53 ± 5.16^{b}	38.96 ± 8.10^{d}	48.93 ± 5.41^{e}	52.81 ± 7.48^{c}
Glucose (mmol/L)	8.7 ± 0.78^{a}	7.55 ± 0.85^{e}	7.35 ± 0.82^{c}	6.86 ± 0.58^{b}	6.8 ± 0.48^{d}
Cholesterol (mmol/L)	1.89 ± 0.30^{a}	1.40 ± 0.32	1.62 ± 0.64^{b}	1.05 ± 0.29^{c}	1.06 ± 0.30^{d}
Triglycerides (mmol/L)	1.76 ± 0.68	1.09 ± 0.28	1.19 ± 0.54	0.75 ± 0.22	1.01 ± 0.22
ALT (μkat/L)	0.13 ± 0.03	0.14 ± 0.03	0.17 ± 0.04	0.19 ± 0.08	0.15 ± 0.05
AST (μkat/L)	0.12 ± 0.07	0.15 ± 0.03^{a}	0.20 ± 0.05	0.11 ± 0.00	0.17 ± 0.07^{b}
ALP (μkat/L)	2.44 ± 0.40	1.94 ± 0.53	1.81 ± 0.63	2.00 ± 0.40	1.81 ± 0.44
Na (sodium, mmol/L)	132.9 ± 2.96	136.1 ± 10.74	136.9 ± 9.72	134.1 ± 7.36	130.5 ± 7.09
K (kalium, mmol/L)	4.60 ±0.44	4.52 ± 0.39	4.03 ± 0.49	3.96 ± 0.18	4.60 ± 0.44
Ca (calcium, mmol/L)	3.30 ± 0.15	3.20 ± 0.47	3.42 ± 0.36	3.16 ± 0.20	3.06 ± 0.22
P (phosphorus, mmol/L)	2.27 ± 0.28	2.23 ± 0.31	2.35 ± 0.28	1.29 ± 0.24	2.04 ± 0.38
Mg (magnesium, mmol/L)	0.80 ± 0.04	0.83 ± 0.09	0.86 ± 0.10	0.82 ± 0.06	0.79 ± 0.09

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Chlorides (Cl, mmol/L)	92.00 ± 3.51	95.94 ± 8.43	93.24 ± 7.28	95.19 ± 6.94	90.63 ± 5.20

Day 0/1, sampling before application, EG/21, experimental group at day 21, CG/21, control group at day 21, EG/42, experimental group at day 42, CG/42, control group at day 42, reference ranges: total proteins, 53-85 g/L, albumine (24-46 g/L), kreatinine (44-141 μmol/L), glucose (5.5-8.6 mmol/L), cholesterol (0.28-2.1 mmol/L), triglycerides (μkat/L) up to 1.44 μkat/L), ALT 0.33-1.19 μkat/L), AST 0.23-0.93 μkat/L, ALP (0.3-2.13 μkat/L), bilirubin μkat/L (4.3-8.5), Na, K (mmol/L), Ca, calcium (2.2-4.2 mmol/L), P, phosphorus (1.2-2.4 mmol/L), CL, chlorides (92-120 mmol/L), Mg, magnesium (0.8-1.20 mmol/L). Kreatinine, $^{ab}p<0.001$, $^{ad}p<0.01$, $^{ac}p<0.001$, $^{ae}p<0.001$, $^{dc}p<0.001$, Bilirubin values in all groups reached less than 0.3 μkat/L, glucose, ^{ca,ce}p<0.001;^{ab,ad}p<0.001, cholesterol, $^{ab,ac,ad}p<0.01$,.

3.3. Hydrolytic Activity, Jejunal Morphometry, Growth Performance, Organic Acids in Chyme, Quality of Meat, Statistical Assessment

Faecal hydrolytic activity measurements are involved in Table 5. The highest values were measured for amylolytic activity at day 0/1, also at day 21 and 42. The values were increased from from day 0/1 in both groups also at days 21 and 42 as well; however, they were higher in EG than in CG and almost stable at days 21 and 42 (Table 5). Xylanolytic activity was also high and increased in both groups at day 21 reaching almost the same values with slight decrease at day 42. The values of pectinolytic activity were only sligthly increased at day 21 comparing to day 21 and they were slightly higher in EG than in CG at days 21 and 42 (Table 5). Celullolytic activity and inulolytic activity reached the lowest values at the start of experiment as well as at day 21; at day 21 they slightly increased but still were balanced. Inulolytic activity was not influenced.

Regarding the jejunal morphometry, stimulating effect of MLS EM 41/3 was noted at day 21 and also after cessation at day 42 when villi height to crypth depth ratio was increased (EG/21, 4. 0 ± 0.16 : CG/21, 3.93 ± 0.98. At day 42, the ratio value was not changed in EG (4. 0 ± 0.16) and in CG it was even decreased (3.86 ± 0.96).

Live weight at the end of experiment in CG was 2671± 335 g and in EG it was 2753 ± 200 g. ADWG in EG at day 42 reached 45.05 g and in CG 39.84 g. Feed conversion was balanced (3. 557 ± 0.378 kg mass per Kg ADWG in CG and 3.662 ± 0.336 mass per Kg in EG). The values evaluated were not significantly changed.

Table 5. Faecal hydrolytic activity in μmoL released products/g dry matter of faeces/min.

	Day 0/1 (*n=10*)	Day 21/ EG (*n=5*)	Day 21/CG (*n=5*)	Day 42/ EG (*n=5*)	Day 42/CG (*n=5*)
Amylolytic activity	3.76 ± 0.53	5.92 ± 0.96	4.60 ± 0.67	5.34 ± 1.00	4.22 ± 0.63
Celullolytic activity	1.68 ± 0.15	2.63 ± 0.56	2.18 ± 0.38	2.04 ± 0.23	2.10 ± 0.46
Xylanolytic activity	3.50 ± 0.39	4.88 ± 0.84	4.23 ± 0.36	3.09 ± 0.52	3.52 ± 1.05
Inulinolytic activity	1.19 ± 0.09	1.64 ± 0.05	1.44 ± 0.29	1.46 ± 0.11	0.91 ± 0.10
Pectinolytic activity	2.66 ± 0.29	3.08 ± 0.63	2.86 ± 0.39	2.84 ± 0.18	2.29 ± 0.58

Day0/1 before application, Day21EG-experimental group at day 21, control group at day 21, EG/42, experimental group at day 42, CG/42, control group at day 42;.

The values of acetic acid were slightly higher in EG than in CG at day 21 (Table 6). At day 42 they were decreased in both groups. The values of other individual organic acids and/or ammonia in caecal chyme were not influenced respectively they were almost in the same values as in EG so in CG (Table 6) at day 21. The pH value was slightly higher in EG than in CG and at day 42 it was not changed in EG. At day 42 were measured lower values of acetic, butyric, caproic and lactic acids (Table 6).

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Table 6. The values of organic acids, ammonia-NH_3 and pH in caecal chyme (mmol/100 mL) at days 21 and 42.

n=4	Day 21/ EG	Day 21/CG	Day 42/ EG	Day 42/CG
Acetic acid	10.65 ± 1.57	12.09 ± 1.82	7.65 ± 2.27	8.03 ± 1.79
Propionic acid	0.47 ± 0.07	0.41 ± 0.08	0.47 ± 0.24	0.62 ± 0.13
Isobutyric acid	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.0	0.02 ± 0.01
Butyric acid	3.20 ± 0.96	2.97 ± 0.67	0.09 ± 0.05	1.65 ± 0.48
Isovaleric acid	0.08 ± 0.05	0.09 ± 0.02	0.05 ± 0.01	2.07 ± 0.76
Caproic acid	0.15 ± 0.06	0.18 ± 0.03	0.06 ± 0.01	0.06 ± 0.01
Valeric acid (mmol/L)	0.12 ± 0.04	0.13 ± 0.02	0.12 ± 0.05	0.01 ± 0.00
Lactic acid (g/100kg)	0.09 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Ammonia (NH_3,mmo/L)	12.49 ± 2.29	12.41 ± 2.10	8.14 ± 1.66	11.76 ± 3.68
pH	6.13 ± 0.09	5.86 ± 0.19	6.01 ± 0.12	6.24 ± 0.34

Day 21/ EG-experimental group at day 21, CG/21, control group at day 21, EG/42, experimental group at ay 42, CG/42, control group at day 42;.

Higher energy values was noted in meat of EG rabbits (Table 7). Measured values were NS.WHC was increased. The other physico-chemical parameters were not negatively influenced.

Table 7. Physico-chemical values in Muscullus longissimus thoracis and lumborum.

n=4	Day 21/ EG	Day 21/CG	Day 42/ EG	Day 42/CG
Water content (g/100g)	74.93 ± 0.35	74.76 ± 0.11	74.29 ± 0.87	74.76 ± 0.31
Protein content (g/100g)	22.89 ± 0.33	22.78 ± 0.19	23.85 ± 0.43	23.22 ± 0.35
Fat content (g/100g)	1.47 ± 0.00	1.42 ± 0.36	1.19 ± 0.15	1.25 ± 0.17
pH 24 p.m.	5.96 ± 0.03	5.99 ± 0.04	5.96 ± 0.05	6.02 ± 0.02
Waster holding capacity g/100g)	26.33 ± 3.32	29.02 ± 4.81	27.08 ± 6.42	27.94 ± 1.03
Energy value (kJ/100g)	443.07 ± 10.75	435.16 ± 9.17	444.48 ± 7.96	436.10 ± 10.19

Day 21/EG, experimental group at day 21, Day 21/CG, control group at day 21, Day 421/EG, experimental group at day 42, Day 42/CG, control group at day 42, NS (not significant), water holding capacity (WHC)

4. Discussion

Assessing the microbiota, the bacterial counts in faeces of broiler rabbits were not influenced by Mundticin-like substance (MLS EM 41/3) application. Bacterial counts in caecum were lower than in faeces except staphylococci and amylolytic streptococci. In appendix were counted almost the same bacterial amounts as in faeces and/or they were found in lower amount. However, picked up bacterial colonies re-isolated from faeces, caecum and appendix and in vitro treated with MLS EM 41/3 were mostly susceptible to MLS EM 41/3. The appendix is related to rabbit immunity via the development of gut associated lymphoid tissue (GALT). It can be enhanced/improved by microbiota which play an important role in rabbit appendix development and diversity in the primary antibody repertoire [7,10].

Enterococci are a group of LAB which constitute a large proportion of autochthonous microbiota in the gastrointestinal tract of human and animals [19]. Many of them can produce antimicrobial active proteinaceous substances-bacteriocins which recently have been involved in group of postbiotics [3,21,22]. In case of up to now described mundticins produced by strains isolated from plants, they showed a limited inhibitory activity mostly against Gram-positive bacteria, listeriae

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involving [22]. In vitro studies with MLS EM 41/3 will continue as well as MLS purification process to have possibility for more concentrated substance use with supposed higher (broader) inhibitory effect.

Hepatal profile in rabbits represented by the enzymes ALT, AST and ALP showed low values meaning that MLS EM 41/3 did not have negative influence on those parameters. ALT is liver specific enzyme in cytoplasm, the level of which is increased in case of hepatal cells membrane damage associated with liver attack. AST is liver unspecific enzyme in mitochondriae and cell cytoplasm as well. This enzyme is also leaked after damage [23]. Natural additives can influence blood parameters depending on the length and dosage of their administration. The hypoproteinemia could be explained by a reduced exogenous protein intake. Similarly as here also after Ent 4231 total proteins were not significantly increased [24]. Hypocholesterolaemic effect of enterocins was also previously noted, e.g., after Durancin ED26E/7 administration and Ent M as well [25]. Mineral profile of rabbits from blood was not influenced and it was in the ranging limit. Some disbalances could be caused by unbalanced mineral content in feed and or sometimes also supposing better intestinal absorption and metabolism of tested minerals in the gastrointestinal tract [25]. Energetic profile involving also glucose values was supported by lantibiotic bacteriocin-nisin but also after Ent2019 [25,26].

In our previous experiments using enterocins, also increase of PA was noted, e.g., after enterocins Ent 2019 and Ent M application ($p<0.01$), and even prolonged stimulation was measured [27]. Moreover, PA was also increased in case of Ent 4231 [24], so independently on producer strains origin of enterocin [27].

On the other side, no oxidative stress stimulation was noted in case of Ent M when the lowest GPx values were noted and lower than in control rabbits, the same situation was also noted after Ent 2019 and Ent 4231 application [24,27**].**

Rabbits health were maintained in good condition during whole experiment. Diet supplementation with bioactive compounds usually produces improved ADWG and feed conversion ratio in rabbits [28] which was also noted after MLS EM41/3 application.

In general, higher hydrolytic activity is detected in young than adult rabbits [29]. Hydrolytic activity in caecum of rabbits after Ent M and ED26E/7 application was high at day 21 and then it decreased. In our case hydrolytic activity was measured in faeces and these values were lower than in caecum as also reported by Pogány Simonová et al. [10]. But these values were higher at day 21 than in day 42. Caecal microbiota and change in their representatives can influence fermentation process in caecum and it is the same also in faeces.

Pogány Simonová et al. [10] presented almost the same pH values after Ent M and Durancin ED26E/7 application in rabbits. Ammonia values in that case were comparable with values in this study at day 21; however, at day 42 they were higher in all groups than in our study (up to 22.,0 g/100 g and up 0.1 in our study). The other organic acid values were almost the same as presented Pogány Simonová et al. [10]. Lactic acid is important for beneficial digestion and also it beneficially influences immunity. It is fully absorbed in rabbit body what could be a reason of low values of LA in rabbits in this study. It is also in association with low caecal amount of LAB detected in caecum. However, coliforms reached up to 10^3 CFU/g and/or 10^4 CFU/g.

Increased tendency in jejunal morphology after MLS EM 41/3 application is in accordance with those results previously reported [27,30,31].

Regarding meat quality, very important parameter is water content which influences ability to water holding capacity, meat colour and digestibility/fragility of meat. These parameters are key points for meat quality mainly during storage. Water content, fat content and water holding capacity are altogether in association. In this study, correlation between the values pH and water holding capacity was found. Energy value is associated with proteins content and fat content. In our case, energy values were higher in meat from EG rabbits. Energy values are important for consumers because that meat is high dietetic with beneficial nutritional and biological value. Energy values in fresh rabbit meat is comparable with red meats [32].

The indigenous intestinal microbiota plays an important role in the regulation of intestinal development and in supporting the host against potential pathogenic microbiota colonization. But

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external factors (diet changes, weaning period which is stressed period) also influences this stability. So, using postbiotics as an innovative feed strategy could have improving impact on digestive immunity and the animals defense capacity (by stimulating phagocytosis) with no negative effect on other parameters which contribute to total health profile of bred animals.

5. Conclusions

Administration of Mundticin-like substance (MLS) EM 41/3 produced by non-autochthonous strain *Enterococcus mundtii* EM 41/3 lead to significant increase of unspecific parameter immunity (PA), while it did not influence microbiota composition with no influence on blood biochemistry. Oxidative stress was not noted, showing higher GPx values in CG than in EG. Moreover, higher weight gain was noted. MLS EM 41/3 stimulated faecal hydrolytic activity and showed no negative influence on rabbit meat quality. Using postbiotic MLS EM 41/3 in feeding strategy looks as contributing factor to improve animal health.

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