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Assessment of Lantibiotic type Bacteriocin-Gallidermin application in Model Experiment with Broiler Rabbits

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Abstract

Gallidermin is a polypeptide-antimicrobial substance of proteinaceous character which belongs to lantibiotic bacteriocins. Broiler rabbits represent a very useful model animal but they also possess tastive meat. After weaning, they are most sensitive to different bacterial infection. Effect of gallidermin has never been tested in animals/rabbits; therefore our testing and results achieved have a novelty character. Forty-eight rabbits, aged 35 days, after weaning, both sexes were divided in the experimental group (E) and the control group (C); 24 animals in each group. Rabbits, meat line M91 fed a commercial granulated diet for growing rabbits. Rabbits in E group received gallidermin (a dose 5 µl-concentration 0.5 mg/ml) per animal/day for 21 days (from day 0/1) in drinking water. Rabbits in C group administered only commercial diet. Experiment lasted 42 days. At day 21, significant reduction of coagulase-negative staphylococci (CoNS) was noted in faeces of E group ($p < 0.05$), coliforms were also reduced. In caecum were significantly decreased enterococci, lactic acid bacteria, CoNS, *Pseudomonas* spp., coliforms at day 21 ($p < 0.001$, $p < 0.05$). Reduction of coliforms was also noted in the appendix. At day 42, significant increase in phagocytic activity was noted in E group compared to rabbits in C group ($p < 0.001$). The others parameters were not negatively influenced.

Introduction

Gallidermin is a polypeptide-antimicrobial active substance of proteinaceous character produced by *Staphylococcus gallinarum* TU 3928. This substance belongs to a group of polycyclic proteinaceous substances called bacteriocins. It contains unusual amino acid residues such as lanthionine, β-methyllanthionine or α, β-didehydroamino acids which are capable to build intramolecular thioether bridges, therefore those group of bacteriocins is called lantibiotics [1]. Jack et al., [2] or Kempf et al., [3] reported the other substances allotted to the Class of lantibiotic bacteriocins, e.g. epidermin, nisin, subtilin, epilancin, Pep 5. Gallidermin is bactericidal mostly towards Gram-positive bacteria indicating a potential advantage for the treatment of human endocarditis, absceses or skin infections, e.g. in the case of multidrug resistant *Staphylococcus aureus* strains [4,5]. Mode of action of gallidermin integrates into plasma membrane, pore forming and inhibition of cells membrane synthesis [6].

Broiler rabbits are useful animal model because of ease handling; but they also have nutritionally acceptable meat [7]. However, after weaning, in short period to receive obligatory feeding, broiler rabbits are most sensitive to different bacterial infection [8]. Our Laboratory of Animal Microbiology (Our Laboratory of Animal Microbiology, Centre of Biosciences of the Slovak Academy of Sciences, v.v.i., Institute of Animal Physiology, Košice, Slovakia) has been focused on bacteriocins-enterocins study for years; especially to study their antimicrobial effect in food-animals. In our previous studies was found e.g. reduction of pseudomonads in faeces of rabbits administered enterocin (Ent) M and also higher average weight gains [9]. Pogány Simonová et al., [10] reported a significant increase in phagocytosis in rabbits with Ent 2019 administration and lower gluthatione-peroxidase (GPx) activity was measured in rabbits receiving Ent M and Ent 2019. Moreover, when lantibiotic bacteriocin-nisin was applied in broiler rabbits, significant decrease in coagulase-positive staphylococci (CoPS) was noted ($p < 0.01$) in faeces of rabbits but also coliforms, pseudomonads and clostridiae were reduced ($p < 0.001$, $p < 0.05$) and also increase in average body weight gain was noted [11]. Up to know, gallidermin has never been tested with the purpose to have *in vivo* antimicrobial effect or beneficial influence in physiological or other parameters. Therefore, our results have an original and a novelty character. Regarding the gallidermin study, only its *in vitro* effect was tested against *Streptococcus pyogenes* and *Str. pneumoniae* strains isolated from children otitis. The growth of those strains was inhibited by MIC 0.09 µg/ml-0.5 mg/ml; expressed in Arbitrary Units it was 200

-3. 200 AU/ml [12]. *In vitro* inhibition effect of gallidermin against biogenic amine-producing faecal staphylococci from ostriches and pheasants was also reported by us recently [13]. In the present study it was studied the effect of a water administration of gallidermin on microbiota, unspecific immunity parameter-phagocytic activity, biochemical profile, growth performance and meat quality in broiler rabbits husbandry.

Materials and Methods

Experimental animals

Forty-eight rabbits, aged 35 days, after weaning, both sexes were divided in the experimental group (E) and the control group (C); 24 animals in each group. Animals included meat line M91 equally divided in E and C groups. All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals approved by the Slovak Veterinary and Food Administration. The experiment was performed in co-operation with our colleagues in Nitra (National Agricultural and Food Centre-NAFC) and it was also accepted by the Ethic Commission of both institutes. Rabbits were kept in standard cages (0.61 m x 0.34 cm x 0.33 m, the type D-KV-72) supplied by Kovobel company (Czech Republic), two animals per cage. The cages allowed the faeces separation. A cycle 16 h of light, 8 h of dark was used throughout the experiment. Temperature 22 °C and humidity 70 ± 5% were maintained throughout the experiment by heating and ventilation systems.

Experimental design and sampling

The animals were fed a commercial granulated diet for growing rabbits as follows (dry matter 847.49g/kg, crude fibre 146.97 g/kg, crude protein 177.99 g/kg) during the experiment with access to water *ad libitum* as previously shown by Chrastinová et al., [14]. Rabbits in E group received additionally gallidermin-$C_{98}H_{141}N_{25}O_{23}S_4$ (Enzo Life Sci corporation, USA, MW 2069.4) in a dose 5 µl (concentration 0.5 mg/ml) per animal/day for 21 days (from day 0/1). This dose was calculated from the analytical *in vitro* test respecting that gallidermin used was pure substance. A stock solution of gallidermin was stored at 4 °C. Prepared gallidermin was applied in the drinking water. Control activity of gallidermin was tested using the agar spot test according to De Vuyst et al., [15] against the principal (the most sensitive) indicator strain *Enterococcus avium* EA5 (isolated from piglet faeces in our laboratory). Based on our previous experiment [9] the volume of water drunk by rabbits was known. The experiment lasted 42 days. Sampling was performed at the start of the experiment (day 0-1) mixture faeces from 48 homogenous animals (from each cage) was collected and mixed in 10 mixture faecal samples (n=10), at day 21 (3 weeks of gallidermin application; from each group (24 animals in each), five mixture samples of faeces from each cage were sampled, n=5), at day 42 (end of experiment, 3 weeks of gallidermin cessation), from each group five mixture samples of faeces from each cage were sampled, n=5). Average initial weight of rabbits was 973.75 ± 102.86 g/957.08 ± 58.41 g respectively.

Rabbits (selected regarding the weight from each cages and group) were slaughtered (n=4) at day 21 and 42, caeca and appendix were sampled for analyses. Rabbits were slaughtered after electro-stunning in an experimental slaughterhouse by cutting the carotid and jugular veins, the bleeding out.

Musculus Longissimus Thoracis (MLT) was separated by the removing the skin and connective tissue, chilled and stored for 24 h at 4 °C until physico-chemical analyses started.

Blood was sampled from *vena auricularis* at days 0-1, 21 and 42 into heparinized and unheparinized Eppendorf tubes depending on the tests.

Media and microbial analyses

To test microbiota, faecal samples were treated using the standard microbiological dilution method; one g of faecal mixtures was diluted in 9 ml of Ringer solution (pH 7.0; Oxoid Ltd., Basingstoke, Hampshire, the United Kingdom). The dilutions (100 µl) were plated on the selective media according to ISO (International Organization for Standardization). To enumerate enterococci M-Enterococcus agar was used (NF-V04503, Difco Laboratories, Detroit, USA). Other lactic acid bacteria-LAB were detected on De Man-Rogosa-Sharpe agar (MRS, ISO 15214, Merck, Darmstadt, Germany). Baird-Parker agar supplemented with egg yolk tellurite solution (ISO 21527-1, Difco) was used to enumerate coagulase-positive staphylococci (CoPS). Coagulase-negative staphylococci (CoNS, ISO 6888) were enumerated on Mannitol salt agar (Becton and Dickinson, Cockeysville, USA). Clostridium agar with the supplement SR0096E and 7% (v/v) of defibrinated horse blood (SR0050, ISO 15883, Oxoid Ltd., Basingstoke, Hampshire, England) was used to enumerate *Clostridium* spp. To count coliforms, Mac Conkey agar (ISO 7402, Oxoid) was used. *Pseudomonas* spp. were isolated on Pseudomonas agar (Biomark, India). The plates were incubated at 30 °C and/or 37 °C for 24-48 h depending on the bacterial genera. Bacterial counts were expressed in colony forming units (log10) cfu per gram (cfu/g) ± SD.

In addition, one g of caecal content and appendix was treated according to the standard microbiological dilution method and plated on the media mentioned above.

Phagocytic activity and biochemical analyses

Blood (n=8 mix from each group) was sampled from *vena auricularis* at days 0-1, 21 and 42. To test phagocytic activity (PA) blood was sampled in Eppendorfs tubes containing microspheric hydrophilic particles (MSHP) and heparin. Direct counting was performed using a modified test described by Vetvička et al., [16] 50 µl of MSH particle suspension (ARTIM, Prague, Czech Republic) was mixed with 100 µl of blood in an Eppendorf tube and incubated at 37 °C for 1 h. Blood smears were then prepared and stained in accordance with May-Grünwald and Giemsa-Romanowski. PA (IPA) was calculated as the number of white cells containing at least three engulfed particles/100 white cells. The percentage of phagocytic cells was evaluated using an optical microscope, by counting PMN up to 100.

Biochemical analyses such as nitrogenous profile (the total proteins, albumin), enzymatic profile (α-amylase, alanine transaminase, [EC 2.6.1.2]), energetic profile (glucose, triglycerides, cholesterol) were tested by the use of analyzator Ellipse (AMS, Italy) and the commercial kits Dialab (Czech Republic). From each group (n=8) samples were used and expressed in the appropriate units ± SEM.

Weight gain and some parameters of *Musculus longissimus thoracis*

MLT quality -pH 24 protein, fat cholesterol (g/100g) and energy value were checked in co-operation with the colleagues in NAFC (Nitra). The ultimate pH was determined using a Radelkis OP-109 (Jenway, England) with a combined electrode penetrating 3 mm into samples. The parameters-content of water, protein, fat were estimated using an INFRATEC 1265 spectroscope; from these values the energy value was calculated: EV(kJ/100g) =16.75x protein content+37.65 x fat content. The water holding capacity was determined by the compress method at constant pressure [17].

Table 1: Microbiota in faeces of broiler rabbits with gallidermin and in control group of rabbits (log cfu/g ± SD).

	E	C
Enterococci		
Day 0-1 (n=10)	4.08 (0.20)	4.08 (0.20)
Day 21 (n=5)	5.46 (0.23)	5.75 (0.39)
Day 42 (n=5)	5.79 (0.24)	5.22 (0.22)
LAB		
Day 0-1	3.74 (0.93)	3.74 (0.93)
Day 21	6.27 (0.25)	6.79 (0.60)
Day 42	6.29 (0.25)	5.42 (0.23)
CoPS		
Day 0-1	4.09 (0.20)	4.09 (0.20)
Day 21	5.04 (0.24)	5.12 (2.26)
Day 42	5.95 (0.24)	5.24 (0.23)
CoNS		
Day 0-1	4.47 (0.11)	4.47 (0.11)
Day 21	4.94 (0.22)a	5.39 (0.52)^{b}
Day 42	5.74 (0.24)	4.39 (0.20)
***Clostridium* spp.**		
Day 0-1	4.54 (0.21)	4.54 (0.21)
Day 21	5.36 (0.72)	5.41 (0.32)
Day 42	5.08 (0.25)	4.84 (0.20)
***Pseudomonas* spp.**		
Day 0-1	5.22 (2.28)	5.22 (2.28)
Day 21	6.50 (2.55)	6.85 (2.61)
Day 42	7.01 (0.26)	6.54 (2.56)
Coliforms		
Day 0-1	2.75 (0.16)	2.75 (0.16)
Day 21	5.30 (2.30)	6.55 (2.56)
Day 42	5.31 (0.23)	5.50 (2.34)

E, the experimental group with gallidermin, C-the control group- the commercial diet only; Day 0-1 of sampling, Day 21 of sampling, 3 weeks of application; Day 42 of sampling, 3 weeks of cessation; LAB-lactic acid bacteria, CoPS, coagulase-positive staphylococci, CoNS, coagulase-nagative staphylococci; In CoNS, E:C, $^{ab}p < 0.05$.

Statistical analysis

Statistical significance was assessed using one-way analysis of variance with the post hoc Tukey multiple comparison test, (one-way Anova). Probability values of less than 0.05 were considered significant. Statistical analyses were performed by t-test ($P<0.05$) ± SD; in the case of biochemical tests ± SEM.

Results

Microbial analysis

Significant reduction of CoNS was noted in faeces of E group compared to C group at day 21 ($^{ab}p < 0.05$) (Table 1). Moreover, slight (mathematical) reduction of coliforms was noted in E group compared to C group (difference 1.25 log cycle). The other microbiota checked in E group were almost in the same counts as in C group; at day 42 even higher counts in E group (LAB, CoNS, *Clostridium* spp. or *Pseudomonas* spp.).

However, at day 21 in caecum of E group, enterococci were significantly differed compared to day 42 ($^{ab}p < 0.001$). Lactic acid bacteria (LAB) in E group were reduced at day 21 compared to day

Table 2: Caecal microbiota in gallidermin group and in control group of rabbits (log cfu/g ± SD).

	E	C
Enterococci		
Day 21 (n=5)	1.05 (0.01)^{a}	1.35 (0.11)
Day 42 (n=5)	2.05 (0.14)^{b}	1.55 (0.12)
LAB		
Day 21	2.50 (0.05)^{a}	2.99 (0.17)
Day 42	3.86 (0.19)^{b}	3.68 (0.19)
CoPS		
Day 21	1.87 (0.13)^{a}	1.89 (0.13)
Day 42	2.24 (0.14)^{b}	1.40 (0.11)
CoNS		
Day 21	1.63 (0.12)^{a}	2.10 (0.14)^{c}
Day 42	4.08 (0.20)^{b}	4.13 (0.23)
***Clostridium* spp.**		
Day 21	5.04 (0.23)	5.16 (0.28)
Day 42	5.50 (0.34)	5.10 (0.25)
***Pseudomonas* spp.**		
Day 21	3.36 (0.18)^{a}	4.41 (0.23)^{c}
Day 42	4.83 (0.19)^{b}	4.47 (0.23)
Coliforms		
Day 21	1.22 (0.01)^{a}	2.70 (0.64)^{b}
Day 42	1.14 (0.01)	1.09 (0.01)

E, the experimental group with gallidermin, C-the control group- the commercial diet only; Day 0-1 of sampling, Day 21 of sampling, 3 weeks of application; Day 42 of sampling, 3 weeks of cessation; LAB-lactic acid bacteria, CoPS, coagulase-positive staphylococci, CoNS, coagulase-negative staphylococci; Enterococci, E21:E42, $^{ab}p < 0.001$; LAB, E21:E42, $^{ab}p < 0.001$; CoPS, E21:E42, ^{ab}difference 0.37 log cfu/g; CoNS, E21:E42, $^{ab}p < 0.001$; E21:C21, $^{ac}p < 0.01$; *Pseudomonas* spp., E21:C21, $^{ac}p < 0.05$; E21:E42, $^{ab}p < 0.01$; Coliforms, E21:C21, $^{ab}p < 0.05$.

42 ($^{ab}p < 0.001$). CoNS were significantly decreased in E rabbits at day 21 and 42 ($^{ab}p < 0.001$) and also at day 21 compared to animals in C group ($^{ac}p < 0.01$). Moreover, at day 21 in E group *Pseudomonas* spp. were reduced compared to C ($^{ac}p<0.05$). They were also decreased in E rabbits at day 21 compared to day 42 ($^{ab}p <0.01$). Coliforms in E group of rabbits were significantly reduced compared to C at day 21 ($^{ab}p < 0.05$). The counts of *Clostridium* spp. in caeca of rabbits were not influenced; CoPS were influenced only slightly in E group at day 21 compared to day 42 (difference 0.37 log cycle) (Table 2).

At day 21 reduction of CoPS was noted in appendix of E rabbits compared to C group, $^{ab}p < 0.01$ (Table 3). Also reduction of coliforms was noted in E group at day 21 compared to C ($^{ab}p < 0.001$) and in E rabbits at day 42 compared to C ($^{cd}p < 0.001$).

Phagocytic activity and biochemical analyses

The initial phagocytic activity (PA) reached 49.33 ± 1.03 % and IPA 2.52 ± 0.10 (Table 4); at day 21, the values of PA reached 51.50 ± 1.23% in E rabbits; in C group it was 51.00 ± 1.41 %. At day 42, significant increase in PA was noted in E group compared to C ($^{ab}p < 0.001$). Comparing PA values in E rabbits at day 21 and 42 with values from day 0-1 indicated that PA was also increased (Table 4).

Table 3: Microbiota in appendix of gallidermin group and control group of rabbits (log cfu/g ± SD).

	E	C
Enterococci		
Day 21 (n=5)	1.73 (0.31)	1.55 (0.01)
Day 42 (n=5)	2.53 (0.15)	1.88 (0.36)
LAB		
Day 21	3.86 (1.96)	4.75 (0.21)
Day 42	4.19 (0.20)	2.29 (0.15)
CoPS		
Day 21	1.91 (0.13)^{a}	2.94 (0.17)^{b}
Day 42	2.48 (0.15)	1.67 (0.12)
CoNS		
Day 21	3.06 (0.75)	3.44 (0.85)
Day 42	4.28 (0.20)	3.83 (0.19)
***Clostridium* spp.**		
Day 21	5.13 (0.27)	5.36 (2.31)
Day 42	6.11 (0.47)	5.79 (0.40)
Pseudomonas spp.		
Day 21	4.72 (0.22)	7.08 (0.27)
Day 42	4.70 (0.21)	4.39 (0.20)
Coliforms		
Day 21	3.48 (0.86)^{a}	5.46 (0.23)^{b}
Day 42	1.41 (0.11)^{c}	2.50 (0.58)^{d}

E, the experimental group with gallidermin, C-the control group- the commercial diet only; Day 0-1 of sampling, Day 21 of sampling, 3 weeks of application; Day 42 of sampling, 3 weeks of cessation; LAB-lactic acid bacteria, CoPS, coagulase-positive staphylococci, CoNS, coagulase-negative staphylococci; CoPS, E21:C21, $^{ab}p < 0.01$; Coliforms, E21:C21, $^{ab}p < 0.001$; E42:C42,$^{cd}p < 0.001$.

Table 4: Phagocytic activity and index of PA expressed in % ± SD.

	PA		IPA	
	Day 21	**Day 42**	**Day 21**	**Day 42**
E	51.50 (1.23)	52.83 (0.75)^{a}	2.80 (0.09)	2.85 (0.05)
C	51.00 (1.41)	49.17 (1.60)^{b}	2.73 (0.10)	2.85 (0.08)

E, the experimental group with gallidermin, C-the control group- the commercial diet only; Day 21 of sampling, 3 weeks of application; Day 42 of sampling, 3 weeks of cessation; Phagocytic activity, PA at day 0-1 was 49.33 % (1.03) and index of PA was 2.52 (0.10); Day 42, E42:C42, $^{ab}p < 0.001$.

The values of the total blood proteins were not influenced (Table 5a). There was even hypoproteinaemia. However, at day 21 higher values of TP were measured in E rabbits compared to C rabbits or compared to day 0-1 ($^{cd,\ bd}p<0.01$) (Table 5a). However, albumin was not influenced; respectively the values were in the physiological range (Table 5a). At day 21 , the albumin values in E rabbits were

Table 5a: Biochemical profile of broiler rabbits.

n=8	**E**	**C**
TP Day 0-1	40.05 (1.88)^{e}	40.05 (1.88)
TP Day 21	47. 31 (4.19)^{a}	36.49 (3.64)^{c}
TP Day 42	49.15 (2.45)^{b}	54.75 (3.24)^{d}
Norm (g/l)	53-85	
Alb Day 0-1	27.82 (0.76)	27.82 (0.76)
Alb Day 21	27.41 (1.24)	23.51 (2.09)^{a}
Alb day 42	29. 46 (1.55)	30.41 (1.37)^{b}
Norm (g/l)	26-46	
ALT Day 0-1	0.14 (0.02)	0.14 (0.02)
ALT day 21	0.17 (0.03)	0.13 (0.03)^{a}
ALT Day 42	0.18 (0.03)	0.21 (0.03)^{b}
Norm (µkat/l)	0.33-1.81	
α-amyl Day 0-1	3.30 (0.45)^{c}	3.30 (0.45)
α-amyl Day 21	2.55 (0.09)^{a}	4.78 (0.41)^{d}
α-amyl Day 42	3.52 (0.22)^{b}	3.94 (0.27)
Norm (µkat/l)	1.88-8.5	
Trig Day 0-1	2.35 (0.09)	2.35 (0.09)
Trig Day 21	2.31 (0.36)	2.18 (0.13)
Trig Day 42	1.91 (0.12)	1.87 (0.15)
Norm (mmo/l)	up to 1.35	
Chol Day 0-1	3.09 (0.19)	3.09 (0.19)^{a}
Chol Day 21	2.58 (0.31)	2.42 (0.12)^{b}
Chol Day 42	2.98 (0.20)	2.70 (0.27)
Norm (mmol/l)	0.28-1.0	
Glu Day 0-1	8.01 (0.16)^{a}	8.01 (0.16)^{d}
Glu Day 21	7.20 (0.56)^{b}	6.61 (0.30)^{c}
Glu Day 42	7.16 (0.12)	7.08 (0.18)
Norm (mmol/l)	5.5-8.6	

E, the experimental group with gallidermin, C-the control group- the commercial diet only; Day 0-1 of sampling, Day 21 of sampling, 3 weeks of application; Day 42 of sampling, 3 weeks of cessation; TP-total proteins; C21:C42, $^{cd}p < 0.01$); C42:E42, $^{bd}p < 0.01$; ^{ae}not significantly influenced; Alb-albumin; C21:C42, $^{ab}p < 0.05$; ALT-alanine aminotranspherase; C21:C42, $^{ab}p < 0.05$; α-amylase; E21:E42, $^{ab}p < 0.01$; E21:0/1,$^{ac}p < 0.01$; E21:C21, $^{ad}p < 0.01$; Chol-cholesterol, E21:0/1,$^{ab}p < 0.05$; Glu-glucose, E21:0/1, $^{ab}p< 0.001$; E21:C21, $^{bc}p<0.001$; C21:0/1, $^{bd}p <0.001$; Trig-triglycerides.

not changed, while in C group they were decreased ($^{ab}p < 0.05$). The values of triglycerides were higher during all experiment compared to physiological value (up to 1.35 mmol/l) and not influenced due to gallidermin. Similarly, hypercholesteraemia was noted; cholesterol values were decreased in both, E and C groups; even reduced compared to sampling 0-1 ($^{ab}p < 0.05$). In general, during all experiment α-amylase values in blood serum were in physiological range, although at the lowest level of the range. However, at day 21 decrease was noted in E rabbits compared to day 0-1 ($^{ac}p < 0.01$) and in E rabbits at day 21 compared to E rabbits at day 42, $^{ab}p < 0.01$) and at day 21 in E compared to C ($^{ad}p<0.01$). The values of Glu were optimized in E at day 21 compared to day 0/1 ($^{ab}P<0.001$) as well as in E compared to C at day 21 ($^{bc}p<0.001$) and in C at day 21 compared day 0/1 ($^{bd}p<0.001$; Table 5a). ALT enzyme was lower than physiological level; in C rabbits even decrease in ALT was noted at day 21 compared to C42 (^{ab} p < 0.05) (Table 5a).

While blood content of P and Mg were in physiological ranges, in the case of Ca hypocalcaemia was noted. However, comparing both groups, at day 21 higher concentrations of minerals in E rabbits than in C were checked (Table 5b) (Ca, E21:E42, $^{ab, ac}p < 0.05$; P, C21:E21, $^{ab}p < 0.001$; C42:E42, $^{cb}p<0.001$, $^{be}p < 0.05$; Mg, E21:E42, $^{ab}p<0.05$); 0 /1:E21, $^{dc}p < 0.05$).

Average daily weight gain (age of slaughter 56 days, growing period 35-56 days was 40.90 respectively 40.93 g/d; during growing period 56-83, at age of slaughter 83 days it was 34.62 g/d in E rabbits and 29. 36 g/d in C group of rabbit; so higher in gallidermin group. No mortality was noted in growing period 35-56 days and during growing period 56-83 days in E group 1 and in C 3 rabbits (n=24).

Water content, water holding capacity, fat, cholesterol, pH 24, energy value listed in Table 6 were not significantly influenced (except energy value in E rabbits-at salughter age 83 days compared to E group at slaughter days 56 ($^{ab}p < 0.05$).

Table 5b: Mineral profile (calcium-Ca, phosphorum-P and Mg-magnesium) in blood serum of broiler rabbits.

n=8	E	C
Ca Day 0-1	1.78 (0.06)^{c}	1.78 (0.06)
Ca Day 21	1.29 (0.13^{a}	1.20 (0.16)
Ca Day 42	1.63 (0.14)^{b}	1.17 (0.11)
Norm (mmol/l)	2.2-4.2	
P Day 0-1	1.07 (0.09)	1.07 (0.09)^{e}
P Day 21	1.64 (0.12)^{a}	0.55 (0.09)^{b}
P Day 42	1.59 (0.10)^{d}	0. 47 (0.11)^{c}
Norm (mmol/l)	0.55-2.13	
Mg Day 0-1	1.06 (0.20)	1.06 (0.20)^{c}
Mg Day 21	1.46 (0.22)^{a}	0.39 (0.13)^{d}
Mg Day 42	0.48 (0.12)^{b}	0.52 (0.20)
Norm (mmol/l)	0.80-1.20	

E, the experimental group with gallidermin, C-the control group- the commercial diet only; Day 0-1 of sampling, Day 21 of sampling, 3 weeks of application; Day 42 of sampling, 3 weeks of cessation; Ca-calcium; E21:E42, $^{ab}p < 0.05$, E21:0/1, $^{ac}p < 0.05$; P-phosphorum; E21:C21, $^{ab}p < 0.001$; C42:E42,$^{cd}p < 0.001$, 0/1:C21, $^{ae}p < 0.01$; Mg-magnesium; E21:E42, $^{ab}p < 0.05$; 0/1:C21,$^{cd}p < 0.05$.

Table 6: Some physico-chemical parameters of meat (n=4).

	Age of slaughter 56 days (day 21)		Age of slaughter 83 days (day 42)	
	E	C	E	C
Water	74.03(0.19)	74.11 (0.12)	74.19 (0.15)	73.97 (0.37)
Protein	23.03 (0.26)	23.31 (0.20)	23.83 (0.14)	23.75 (0.17)
Fat	0.86(0.19)	0.67 (0.12)	1.21 (0.20)	0.75 (0.87)
Chol	0.24 (0.06)	0.22 (0.05)	0.23 (0.07)	0.25 (0.06)
pH24	5.72 (0.04)	5.73 (0.02)	5.47 (0.15)	5.47 (0.15)
Whc	36.09 (3.50)	36.09 (3.50)	31.66 (1.87)	31.66 (1.87)
EV	419.13 (6.54)^{a}	415.64 (4.83)	444.57 (7.55)^{b}	430. 41 (7.21)

E, the experimental group with gallidermin, C-the control group- the commercial diet only; Day 0-1 of sampling, Day 21 of sampling, 3 weeks of application; Day 42 of sampling, 3 weeks of cessation; water (g/100g), protein (g/100g), fat (g/100g), cholesterol (g/100g), water holding capacity; energy value (kJ/100g) ; EV: E21:E42, $^{ab}p < 0.05$.

Discussion

In vitro inhibition effect of gallidermin against staphylococci was reported in literature (Pag and Sahl [6]); this was also confirmed *in vivo* in our experiment. Moreover, randomly picked up colonies from Baird-Parker agar were submitted to *in vitro* treatment with gallidermin and their growth was inhibited (unpublished data). In some cases it could be disputed that in our experiment, bacteriostatic effect of gallidermin on microbiota of broiler rabbits was demonstrated; because no pressure or interaction was there acted, the bacterial strains increased. The best reduction effect of gallidermin was noted in caecum of broiler rabbits. As already reported in our previous studies, antimicrobial effect of lantibiotic-nisin as well as enterocins against spoilage bacteria was reported in rabbits husbandry [9,18]. Surprisingly, *in vivo* decrease of faecal coliforms, staphylococci, enterococci and pseudomonads was noted in rabbits even after nisin application. As resulted, coliforms, staphylococci and pseudomonads were decreased also after gallidermin application. Gallidermin belongs to quite stable bacteriocins; in spite of this, conditions of the gastrointestinal tract can influence its antibacterial effect.

Similarly as in our previous experiments [9,18] by using enterocins (e.g. Ent M, Ent 4231) or lantibiotic bacteriocin nisin prolonged stimulation of phagocytosis was noted. After administration of lantibiotic nisin in rabbits at days 21/28 and 42, lower PA values were measured (E21/28-41.00 ± 1.79%:C21-30.00 ± 2.10; E42-41.67 ± 0.66%:C42-29.83 ± 1.974) compared to the values of PA in experiment with gallidermin; at day 42 PA value in blood of E rabbits reached 52.83 ± 0.75% and in C rabbits it was 49.17 ± 1.60%; p<0.001. It is at first time (done by our group) when effect of lantibiotic on PA in animals/rabbits was demonstrated. Moroever, stimulation of PA was also repeatedly referred in broiler rabbits after enterocins application; after e.g. Ent M application, PA value reached 68.8 ± 0.86%; even higher than after application of Ent M-producing strain AL41 (44.8 ± 0.66%). The stimulating effect of bacteriocins regarding the immunity can be associated with increased macrophage PA. Granulocytes are responsible for the non-specific immune response and in the first line share phagocytosis introdefence of the host to infectious and inflammatory actions [19] it means that enhanced polymorphonuclear

cell activity can contribute to an increased ability to combat microbial infections as was also measured after application of Ent M-producing, probiotic strain *Enterococcus faecium* AL41=CCM8558 in broiler rabbits or broiler chickens/hens [9,19]. Pogány Simonová et al., [10] reported a significant improvement of the digestive immunity and the hosts defense capacities by stimulating leucocyte phagocytosis after enterocin Ent 2019 application. It can be supposed that by enterocins stimulating PA can act through the modulation of gut microbiota favoring lactic acid bacteria and due to supporting the GALT by the stimulation of the IgA system [20]. Moreover, the prolonged immuno-stimulative effect of enterocins should be also explained by the stimulation of GALT and IgA secretion [21].

Regarding the energetic profile (glucose, triglycerides and cholesterol), these parameters were in the range of reference values. Hypoproteinaemia was noted in E group of rabbits; which however, is not accompanied by albumine decrease; it indicates reduced exogennic addition of proteins than their reduced synthesis or lost. In rabbits was noted hypokalcemia; in spite of E rabbits, in rabbits of C group, Ca was increased at day 21. For rabbits high quality hay is a main source of Ca; lack of hay in rabbits can be explained by winter season diet. Activity of liver enzyme ALT was not influenced; it is similar as it was in hens after probiotic product application which included four lactobacilli, one *Enterococcus faeicum* and one *Streptococcus thermophilus* [22].

Weight gain and parameters of MLT were not influenced by gallidermin application; their values are comparable with the values achieved after application of enterocin M or durancin-like [14,18,21].

Conclusion

It can be assessed that gallidermin showed antimicrobial effect on microbiota in rabbits as well as it stimulated PA. These results possess a novel character and they can be interesting also for breeders because pure substance of gallidermin was used, this means small concentration only is enough effective which could be promising from financial aspect.

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