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USE OF ENTEROCIN M SUBSTANCE APPLIED IN DRINKING WATER AND NATURAL ZEOLITE AS DIETARY SUPPLEMENTS FOR GROWING RABBITS

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ABSTRACT

A total number of 72 post-weaned rabbits (aged 35 days, meat line M91 a P91 hybrid rabbits), reared in an intensive breeding in the Slovak Republic, were used in the experiment. The rabbits were randomly divided into 3 similar groups, 24 animals in each group (control -EG1, experimental groups – EG2 and EG3) and kept in standard metal cages, two animals per cage. This study investigated the effects of different feeding on growth performance, chemical composition, fatty acid profile and content of essential amino acids of the *Musculus longissimus thoracis* and *lumborum* of growing rabbits. This *in vivo* study was designed to reveal whether the antimicrobial effect of Enterocin M (produced by non-rabbit origin strain *Enteroccocus faecium* AL41-CCM8558), is able to influence the meat quality of rabbits. Every day at the same time in the morning the rabbits in EG 2 received 50 µl dose of Ent M per animal per day, administered into their drinking water. The rabbits of experimental group (EG3) had commercial diet enriched with the supplement of 1 % natural zeolite (a product of ZEOCEM Company, Bystré, Slovakia) and the drinking water did not contain any coccidiostatic drugs during the experiment. The experiment was lasted for 42 days.
The fat of rabbits in the 2EG had a higher ($P < 0.05$) concentration of n-3 and n-6, MUFA, while the ratio of SFA in the control was lower (32.92 %) than in 2EG and 3EG reared rabbits (34.61 % vs. 34.71 %). Feeding of natural substances to rabbits did not influence biochemical and zootechnical parameters and it had no negative effect on growth performance in rabbits. It had positive effect on health status and it reduced the number of *Eimeria* spp. Oocysts were not detected in rabbits' intestinal tract.

Key words: rabbit meat; enterocin; zeolite

INTRODUCTION

In recent years, naturally occurring antimicrobial and antioxidant compounds have been preferably employed in meats because of their potential health benefits and safety compared to synthetic preservatives (Lauková *et al.*, 2015, 2016; Strompfová *et al.*, 2017). Among those compounds/substances enterocins have proteinaceous character and produced mostly by the representatives of the genus *Enterococcus*. Enterocins can inhibit the growth of both Gram-positive and Gram-negative bacteria (Franz *et al.*, 2007) and were used with beneficial influence on rabbit husbandry (Lauková *et al.*, 2012).

Zeolite is a substance of crystal structure, containing cations of alkaline-earth metals. Zeolite

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is used to modify fermentation processes including buffers, which regulate gut pH and favour the activity of cellulolytic bacteria, compounds to suppress methane production and bloat-preventing compounds, which prevent the build-up of gas trapped in foam (CO_2, NH_3, H_2S, CH_4 and some nitrogen combinations) in the caecum fluid (Shibaev and Butko, 1986; Shadrin, 1988). Because breeders have been looking for ways to maintain or improve animal health status in association with meat production, they are more open to use natural substances.

The aim of this *in vivo* study was to determinate the effect of Enterocin (Ent) M, applied to drinking water and zeolite, as dietary supplements for growing rabbits, on growth performance, selected physico-chemical parameters and nutritional quality of rabbits meat.

MATERIALS AND METHODS

A total number of 72 post weaned rabbits (aged 35 days, meat line M91 a P91 hybrid rabbits), reared in an intensive breeding in the Slovak Republic, were used in the experiment. The rabbits were randomly divided into 3 similar groups (control-EG1, experimental group EG2, experimental group-EG3), 24 animals in each group, and kept in standard metal cages, two animals per cage.

A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Temperature and humidity in the building were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed the building air temperature to be maintained within 21 ± 4 ˚C throughout the experiment. Relative humidity was about 70 ± 5 %. The rabbits were fed a commercial diet (KV; TEKRO Nitra, Ltd. Slovak Republic) pellets of 3.5 mm in diameter *ad libitum* and water was provided *ad libitum* using nipple drinkers. The experiment was performed in co-operation with the Institute of Animal Physiology, Centre of Biosciences of the Slovak Academy of Sciences in Košice. All care and experimental procedures were approved by the Slovak Veterinary and Food Administration and by the Ethic Commission of both institutes. Every day at the same time in the morning the rabbits in EG2 received 50 µl dose of Ent M per animal per day, administered into their drinking water. The rabbits of the experimental group (EG3) were fed a commercial diet enriched with supplement of 1 % natural zeolite, and the drinking water did not contain any coccidiostatic drugs during the experiment. The zeolite (ZeoFeed) was supplied by Zeocem Company (Zeocem a.s., Bystré, Slovakia) from the quarry of Nižný Hrabovec, Slovakia. Zeolite used in the experiment had a particle size of 0.2 – 0.5 mm and contained more than 80 % of clinoptilolite, as determined by X-ray powder diffraction. Its chemical composition was as follows: SiO_2 – 70.98 %, Al_2O_3 – 11.72 %, Fe_2O_3 – 1.26 %, CaO – 2.89 %, MgO – 0.53 %, K_2O – 3.25 %, Na_2O – 0.56 % and loss on ignition – 7.17 %.

To detect *Eimeria* sp. oocysts, the quantitative McMaster method (1986) was used; the oocyst counts were expressed in OPG.g^{-1} (detected oocysts per gram of faecal sample).

The ingredients and chemical composition of this diet is presented in Table 1, according to procedures of the AOAC (2005) and Van Soest *et al.* (1991).

The body weight of each experimental animal was recorded weekly during the whole study. Weight feed mixture was checked daily and average daily weight gain and feed conversion were calculated mathematically, as well as mortality, at the end of the experiment. Five animals (at the age of 70 days) from each group were slaughtered and samples were taken. After electro-stunning (90 V for 5 s), rabbits were slaughtered in an experimental slaughterhouse by cutting the carotid and jugular veins and bleeding out. *Musculus longissimus thoracis* and *lumborum* (*MLTL*) was separated by removing the skin and connective tissue chilled and stored at 4 °C for 24 h until physico-chemical analysis started. The pH value was determined after 24 h (*post-mortem*) using a Radelkis OP-109 measure device (Jenway, England) with a combined electrode penetrating 3 mm into samples. The electrical conductivity (µS.cm^{-1}) defined as locations of muscles were evaluated using PMV 51 (Tecpro GmbH, Germany), colour characteristic were expressed by CIE L*a*b system (lightness-L*, 0: black and 100: white), (redness and greenness-a*; yellowness and blueness-b*) using a Lab. Miniscan, Lightness measurements at room temperature were also performed. Physico-chemical characteristics and

Table 1. Composition and nutrient content of granulated diet for growing rabbits (g.kg^{-1} in original matter)

Ingredients	%	Chemical analysis	Control diet	Control diet + 1 % Zeolite
Lucerne meal	36.0	Crude protein (N*6,25)	187.74	197.96
Extracted sunflower meal	5.5	Crude fibre	170.15	180.43
Extracted rapeseed meal	5.5	Fat	34.24	36.05
Wheat bran	9.0	Ash	67.09	81.06
Oats	13.0	Starch	127.76	95.19
Malt sprouts	15.0	Organic matter	826.65	803.65
DDGS	5.0	ADF	203.17	210.79
Sodium chloride	0.3	ADF	349.46	321.06
Mineral and vitamin mixture*	1.7	Calcium	9.09	8.60
Barley grains	8.0	Phosphorus	5.74	6.50
Limestone	1.0	ME (MJ.kg^{-1})	10.95	10.82

*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, 5 000 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, 4 000 mg, choline chloride, 100 000 mg. DDGS: dried distillers grains with solubles; ADF: Acidodetergent fibre; NDF: Neutraldetergent fibre; ME: metabolisable energy

chemical composition were determined by standard methods (STN 570185). The content of water, protein and fat were estimated using a FoodScaneTM – Meat Analyser (FOSS, Denmark) by a FT IR method (Fourier Transform infrared Spectroscopy); expressed in g.100 g^{-1}. From these values, the energy value was calculated according to the equation of Strmiska *et al.* (1988):

Energy value (kJ.100 g^{-1}) = 16.75 x protein content + 37.65 x fat content.

The water holding capacity was determined by the compress method at constant pressure (Hašek and Palanská, 1976; Rafay *et al.*, 2008). The analysed 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 a pressure of 5 kg for 5 min. The results were calculated

Table 2. Effect of treatment on performance of rabbits (mean ± SD)

Parameter (n = 24)	EG1 control	EG2 control + Ent M	EG3 control + 1 % Zeolite
Number of animals in groups	24	24	24
Initial live weight (35 d), g	1059 ± 99	1000 ± 98	1153 ± 136
Intermediate live weight (56 d), g	1981 ± 207	1953 ± 164	2077 ± 237
Final weight (77 d), g	2779 ± 300	2635 ± 334	2832 ± 264
Feed conversion ratio between 35th and 56th day (g.g^{-1})	3.466 ± 0.354	3.434 ± 0.347	3.537 ± 0.287
Feed conversion ratio per kg gain	3.882 ± 0.320	3.769 ± 0.461	3.849 ± 0.258
Mortality (n)	4	4	4
Daily weight gain, (g.d^{-1})	40.95	38.93	40.38
Carcass value (%)	55.67 ± 1.41	55.36 ± 1.05	55.42 ± 1.15

P > 0.05; not significant differences from control; EG-experimental group

from the difference in weight between the slips with aspirating spot and the pure filter paper. The ash content was determined by mineralization of the samples at 550 °C according to STN 570185. The fatty acid (FA) composition of MLD samples was determined by the method of Ouhayoun (1992) and Bannon *et al.* (1982) by gas chromatography of fatty acid methyl ester (FAME) on GC 6890N (Agilent Technologies, Switzerland). The results were expressed as percentages of total fatty acids. Fatty acid composition varies a lot and it is expressed as share of SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acids), P/S and n6/n3 index. The amino acid composition of the diet was analysed by an ion-exchange chromatography on AAA (Ingos Prague, Czech Republic) after acid hydrolysis with 6M HCl and methionine and cystine after oxidation hydrolysis.

The results were expressed as the mean ± SD. Mean values within the same row having different superscripts indicate significant difference using Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Among the experimental groups no significant differences were found in feed intake, feed conversion ratio and carcass value in the fattening experiment. Furthermore, rabbits reared in the EG3 group weighed more but the difference was not significant. The average daily weight gain was lower in the both experimental groups comparing to the control group: EG1 – 40.95 g, EG2 (*Ent* M) – 38.93 g, EG3 (1 % zeolite) – 40.38 g.

Table 3. Physio-chemical characteristics of rabbit meat (*MLTL*) 24 h *post-mortem* (n = 5)

Characteristic Age at slaughter (70d)		EG1 control		EG2 control + Ent M		EG3 control + 1 % Zeolite	
		mean	SD	mean	SD	mean	SD
Water	$g.100g^{-1}$	74.41	0.43	74.18	0.62	74.72	0.26
Protein	$g.100g^{-1}$	23.19	0.19	22.98[a]	0.14	23.11	0.19
Fat	$g.100g^{-1}$	1.16	0.26	1.30[a]	0.26	1.16	0.16
Collagen fibre	$g.100g^{-1}$	0.89	0.18	0.77	0.16	0.81	0.05
Energy value	$kJ.100g^{-1}$	432.07	12.48	433.83	8.04	430.58	5.75
Water holding capacity	$g.100g^{-1}$	23.02	4.81	24.19	3.77	29.09	6.16
Ash	$g.100g^{-1}$	0.62	0.15	0.57	0.14	0.53	0.10
Cholesterol	$g.100g^{-1}$	0.38	0.03	0.36[a]	0.036	0.39	0.02
pH 24		6.05	0.05	5.99	0.06	5.96	0.05
* Colour L	L	50.66	1.25	48.24	2.57	50.01	3.76
Electric conductivity	$\mu S.cm^{-1}$	1.08	0.26	1.51[a]	0.27	1.01	0.59
	Fatty acid composition in intramuscular fat (% of total fatty acids)						
SFA	%	32.924	1.135	34.606	1.760	34.710	1.710
MUFA	%	47.862	1.152	48.176[a]	0.351	47.180	0.681
PUFA	%	13.224	1.195	12.280	1.210	12.228	0.769
Essential FA	%	9.236	0.091	8.178[a]	0.863	8.913	0.520
ω 6	%	8.676	0.521	9.012[a]	0.847	8.670	0.656
ω 3	%	0.442	0.026	0.452	0.030	0.440	0.037

[a] $P < 0.05$ significant differences from control; EG-experimental group

SFA: saturated fatty acids, include C8:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0.

MUFA: monounsaturated fatty acids, include C16:1 n-7, C18:1 n-9c, C18:1 n-9t, C20:1, C22:1.

PUFA: polyunsaturated fatty acids, include C18:2 n-6, C18:3 n-3, C20:4 n-6, C20:5n-3, C22:5 n-6, C22:6n-3.

The feed conversion ratio also decreased in the groups administering enterocins: in the EG2 group (*Ent* M) – 3.434; in the EG3 (1 % zeolite) – 3.537. These findings are in agreement with our previous results (Pogány Simonová *et al.*, 2015; Szabóová *et al.*, 2011; Lauková *et al.*, 2016). Results regarding the growth parameters are shown in Table 2. After Ent M applying into the water (50 µl per animal per day for 6 weeks), growth performance of rabbits and physio-chemical parameters of meat quality and nutritional value were influenced. Moreover, also non-autochthonous strain can have protective and beneficial effect on broiler rabbits (Lauková *et al.*, 2012; 2016; Strompfová *et al.*, 2017; Chrastinová *et al.*, 2018). No significant differences were observed in the mean carcass yield ratio, the weights of liver, heart and kidney in the tested variants. *Eimeria* sp. oocysts were not detected in the faecal samples of rabbits.

The selected meat quality parameters (content of water, content of proteins, fat, content of amino acids and fatty acids profile, the electric conductivity, ultimate pH 24 (*p.m.*), colour meat characteristic) are presented in Table 3. The oral administration of Ent M lead to a significant increase in the electrical conductivity, compared to control, and to decrease in cholesterol contents ($P \leq 0.05$) of the MLTL. The fat of rabbits in the 2EG had a higher concentration of n-3 and n-6 and MUFA ($P < 0.05$), while the ratio of SFA in control was lower (32.924 %) than in 2EG and 3EG groups of reared rabbits (34.61 % vs. 34.71 %).

Meat is a major source of proteins, essential amino acids, minerals and fatty acids. Protein and lipid contents of meat are closely related to the energy value (Dalle Zotte, 2002; Dalle Zotte and Szendrö, 2011; Pogány Simonová *et al.*, 2015). Chemical composition of meat is closely related to the age. This results are in agreement with the studies investigating effects of age and genotype on muscle composition (Gondret *et al.*, 1998; Chrastinová *et al.*, 2010; Combes, 2004; Bianch *et al.*, 2006; Kalafová, *et al.*, 2014; 2018). The amino acid composition in *MLTL* muscle is shown in Table 4. The essential amino acid composition is one of the most important nutritional qualities of protein.

Only slight differences were found between the individual components, which corresponds with the results of the other authors. Dietary supplementation with 1 % natural zeolite reduced feed conversion ratio, but differences were not significant ($p > 0.05$).

CONCLUSION

Feeding natural substances to rabbits did not negatively influence biochemical and zootechnical parameters and it also had no negative effects on growth performance in rabbits. It had a beneficial effect on health status and reduced the number of *Eimeria* spp. Oocysts were not revealed in the rabbit intestinal ecosystem. In this way, rabbit meat could also be considered to be a functional food.

Table 4. The content of essential amino acids in *MLTL* muscles 24 h *post-mortem* of rabbits (g.100g^{-1}) tissue

Characteristic (n = 5)	EG1 control	EG2 control + Ent M	EG3 control + 1 % Zeolite
Threonine	0.783 ± 0.068	0.709 ± 0.148[a]	0.742 ± 0.057
Valine	0.792 ± 0.053	0.758 ± 0.118	0.767 ± 0.051
Methionine	0.549 ± 0.038	0.514 ± 0.099	0.520 ± 0.032
Cystine	0.231 ± 0.012	0.214 ± 0.034[a]	0.222 ± 0.003
Isoleucine	0.703 ± 0.080	0.635 ± 0.163	0.654 ± 0.075
Leucine	1.405 ± 0.133	1.291 ± 0.293[a]	1.321 ± 0.120
Phenylalanine	0.728 ± 0.065	0.676 ± 0.147[a]	0.687 ± 0.057
Histidine	0.702 ± 0.053	0.665 ± 0.147[a]	0.680 ± 0.054
Lysine	1.529 ± 0.160	1.397 ± 0.342[a]	1.428 ± 0.142
Arginine	1.151 ± 0.122	1.049 ± 0.257[a]	1.073 ± 0.109

[a] $P < 0.05$ significant differences from control

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