ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2019 Vol. 62, Št. 2: 15–25

Effect of *per oral* administration of the ŽP strain, a new potential probiotic, on rats

Učinek seva ŽP, novega potencialnega probiotika, po peroralnem vnosu na podgane

Irina L. Maslennikova[1], Ekaterina G. Orlova[1], Julia S. Gizatullina[1], Marjanca Starčič Erjavec[2], Natalia P. Loginova[3], Yana N. Troinich[3], Marina V. Kuznetsova[1,3*]

[1] Institute of Ecology and Genetics of Microorganisms Ural Branch Russian Academy of Sciences – branch of Perm Federal Research Center Ural Branch Russian Academy of Sciences, Perm, Russia
[2]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[3]Perm State Medical University named after Academician E. A. Wagner, Perm, Russia
*correspondence: mar@iegm.ru

Abstract: Bacterial antimicrobial resistance is worldwide rising and is already reaching worrisome levels, hence WHO released the priority bacterial pathogen list for research and development of new antimicrobial agents. ŽP strain, a new Nissle 1917 based antimicrobial strain harbors a bacteriocin, colicin ColE7, bacterial conjugation-based "kill" – "anti-kill" antimicrobial system. In previous studies the efficiency of ColE7 bacterial conjugation-based antimicrobial system was shown against different *Escherichia coli* strains. In this study its effect after the *per oral* administration of the ŽP strain, on the host, namely on rats of both sexes was studied. The following parameters were analyzed weight, histology of intestine, spleen and Peyer's patches, blood biochemistry, hematology and microbicide activity of leukocytes and peritoneal macrophages. Our results showed that when administered at a dose of 5×10^8 CFU in the drinking water the ŽP strain is safe and responsible for control and maintenance of an adequate local and non-specific immune response and can also improve the performance of animals in terms of weight gain. Hence our results indicate that it is a promising probiotic.

Keywords: probiotic, Nissle 1917 strain, host response, rat

Izvleček: Odpornost proti bakterijskim protimikrobnim zdravilom narašča po vsem svetu in že dosega zaskrbljujočo raven, zato je WHO objavil prednostni seznam bakterijskih patogenov za raziskave in razvoj novih protimikrobnih zdravil. Sev ŽP, nov antimikrobni sev na osnovi seva Nissle 1917 vsebuje na konjugaciji temelječ protimikrobni dejavnik, ki ga sestavljata gen "kill" (gen za bakteriocin, kolicin ColE7) in gen "anti-kill" (gen za imunost proti kolicinu E7). V prejšnjih raziskavah je bila učinkovitost tega na konjugaciji temelječega protimikrobnega sistema dokazana že na različnih sevih bakterije *Escherichia coli*. V tej študiji smo preučevali učinek tega seva po peroralni uporabi na gostitelja, in sicer na podgane obeh spolov. Parametri, ki smo jih preučevali so bili masa živali, histologija črevesja, vranice in Peyerjevih ploščic, biokemija krvi, hematologija in mikrobicidna aktivnost levkocitov in makrofagov. Naši rezultati so pokazali, da je sev ŽP, če ga dajemo v odmerku 5×10^8 CFU v pitni vodi, varen in ima imunostimulacijski učinek ter lahko tudi izboljša status podgane v smislu povečanja telesne mase. Zato naši rezultati kažejo, da gre za obetaven probiotik.

Ključne besede: probiotik, sev Nissle 1917, odziv gostitelja, podgana

Introduction

In December 2014 a report was published with the estimation that by the year 2050 there will be worldwide 10 million death cases due to antimicrobial resistance (O'Neill 2014). In 2017 World Health Organization released the priority bacterial pathogen list for research and development of new antimicrobial agents (WHO 2017). On this list in the group of bacteria designated with the highest priority for research and development of new antimicrobial agents there are also carbapenem-resistant and ESBL-producing *Enterobacteriaceae*. Since the bacterium *Escherichia coli* (*E. coli*) belongs to the family of *Enterobacteriaceae* and several different carbapenem-resistant and/or ESBL-producing strains were already isolated from clinical and agricultural samples (Ewers et al. 2012, Silva et al. 2012, Allocati et al. 2013, Albiger et al. 2015, Koga et al. 2015, Boulianne et al. 2016, Karlowsky et al. 2017, van Duin and Doi 2017, Davis et al. 2018), it is also crucial to find new antimicrobial agents targeting *E. coli*.

A new antimicrobial agent was constructed with modifications of the well known *E. coli* probiotic Nissle 1917 strain by Starčič Erjavec et al. (2015). The authors introduced the conjugative plasmid pOX38 (a derivative of the F-plasmid) carrying the "kill" gene (ColE7 DNase activity gene) and a chromosomally encoded "anti-kill" gene (ColE7 immunity gene). Action of this special antimicrobial agent (ŽP strain) is based on the conjugative transfer of the "kill" gene into a recipient cell, where it is expressed and the recipient killed. Acquisition of resistance to ColE7 in this system is excluded. The efficiency (killing activity) of the ŽP strain was already tested in several different types of conjugation assays with different *E. coli* recipients (Starčič Erjavec et al. 2015, Maslennikova et al. 2018).

According to literature the probiotic effect of the original Nissle 1917 strain is based on its local intestinal anti-inflammatory effects due to activation of antimicrobial agents of mucosal epithelium, inhibition of adherence and invasion of other bacteria and its influence on systemic and non-specific immune response (Wehkamp et al. 2004, Arribas et al. 2009). We proposed that the new ŽP strain, which is based on the *E. coli* Nissle 1917 strain, will positively combine the newly acquired property of killing *E. coli* recipients and the positive properties of the original strain. It can be assumed that the ŽP strain is safe to be used as a probiotic, however experiments confirming this assumption are needed.

The aim of this study was to analyze the effect of *per oral* administration of the ŽP strain on survival, weight, morphology of intestinal epithelium, spleen and Peyer's patches, and hematological and biochemical parameters of peripheral blood and microbicide activity of leukocytes and peritoneal macrophages of rats of both sexes.

Material and methods

Animals

Male and female rats (Wistar line) age 4 weeks were used. Rats were housed in groups of five rats per cage. All animals were maintained in a room with a regular 12-h light/dark cycle at 20±2 °C. Before the experiment, the animals received conventional balanced diet and water *ad libitum*. All animal protocols were pre-approved by the Ethical Committee of Perm State Medical University named after academician E.A. Wagner (PSMA). All experiments complied with the current laws of Russia: GOST 34088-2017.

Bacterial strain

ŽP strain (*E. coli* Nissle 1917 harboring the pOX38 with the "kill" gene (ColE7 activity gene) and a chromosomally encoded "anti-kill" gene (ColE7 immunity gene) was used throughout the study.

Overnight cultures were grown in sterile Luria-Bertani (LB, USA) broth at 37 °C. After incubation, cells were harvested by centrifugation at 5000 g for 10 min (Centrifuge 5804, Eppendorf,

Germany) and resuspended in sterile NaCl (0.9%). The strain was administered to rats each morning at a concentration of 5×10^8 or 5×10^{10} colony forming units (CFU) per drinking water bottle per cage from Day 1 till Day 21.

Experimental rat groups

Male and female rats were further subdivided into three experimental groups: (1) control group receiving the main diet and sterile drinking water in a 400-ml drinking water bottle; (2) the experimental group receiving the main diet + 5×10^8 CFU of ŽP strain per a 400-ml drinking water bottle; (3) the experimental group receiving the main diet + 5×10^{10} CFU of ŽP strain per a 400-ml drinking water bottle. All animal groups received food in equal proportions *ad libitum* during experiment.

Animal's body weights were measured twice (first time on Day 1 and the second time on Day 21) during the experiment. At Day 21, rats from all groups anesthetized by short-term narcosis were sacrificed by decapitation and samples of blood, serum, intestine, Peyer's patches, and spleen were taken for analysis.

Histologic analysis

Samples of the intestine, Peyer's patches, and spleen were fixed in 10% neutral formalin in phosphate buffer (pH 7.2) and poured into «Histomix» paraffin (BioVitrum, Russia). Sections were stained with hematoxylin and eosin to evaluate the overall morphological picture.

Blood analysis

From each animal included in the study blood samples after sacrificed by decapitation was collected on Day 21. For the biochemical blood analysis, a 2 ml of blood sample was centrifuged at 1000g for 10 min (Centrifuge 5415R, Eppendorf, Germany). The following biochemical serum parameters: total protein (TP), glucose (Glu), total cholesterol (TC), calcium (Ca), phosphorus (P), creatinine (Crea) and urea (Urea), were detected using a chemical analyzer MINDRAY BS 200 (China).

For analysis of absolute blood cell count, Hb concentration the automated Hematological Analyzer (DREW-3, Drew Scientific, USA) was used.

Ex vivo microbial killing and chemiluminescence assay

For the *ex vivo* microbial killing and chemiluminescence assay peripheral blood leukocytes and peritoneal macrophages obtained from each animal were used.

To obtain the leukocyte suspension, the following sedimentation method was used: heparin (50-100 units per ml of blood) was added to 1-3 ml of animal blood sample and diluted twice with Hank's solution without phenol red (Biolot, Russia) and mixed with 0.2% methylcellulose (Sigma-Aldrich, USA) in a Hank's solution in the ratio 1:1. One ml of prepared suspension was transfer to fresh tubes and incubated at room temperature for 15-30 minutes. After sedimentation of red blood cells (RBC), the leukocyte suspension was transferred to a fresh tube and washed twice with Hank's solution. The centrifugation was carried out at 300 g for 8 min (Centrifuge 5804, Eppendorf, Germany).

The peritoneal cells were isolated by washing the abdominal cavity with 10 ml of Hank's solution supplemented with 20 U/ml of heparin, 2 mM L-glutamine and 10 mM HEPES (Sigma-Aldrich, USA). The obtained cells suspension was washed twice with Hank's solution as stated previously for leukocyte suspension.

Ex vivo microbial killing and luminol-dependent chemiluminescence assay of leukocytes of peripheral blood and peritoneal macrophages from abdominal cavity was evaluated by inhibition of bacterial luminescence according the published protocol (Maslennikova et al. 2017).

Statistical analysis

Data were expressed as mean ± SD. The normality of data distribution was tested using the χ^2-test. The results indicated that the distribution of the variable was normal. Data were analyzed using the *U*-test. A probability level of $p < 0.05$ was considered significant.

Results and discussion

Effect of ŽP strain administration on the survival and weight of the rats

In order to assay the effect of ŽP strain on the animals, initially the effect of the ŽP on the survival and weight of the employed animals was determined. As seen from Table 1 all the animals survived till Day 21, when the experiment ended. Compared to the control group the average weight (g) of animals of both sexes, which received the ŽP strain in drinking water, increased for at least 2%.

Earlier Aboderin and Oyetayo (2006) reported a significant increase in the weight gain by rats fed culture of *Lactobacillus plantarum* when compared to the control. In agricultural use of probiotics an increase of 1-3% is already considered to be significant (Timmerman et al. 2006, Mountzouris et al. 2007, Matusevicius et al. 2010), therefore it can be concluded that the strain ŽP has a positive effect on weight gain of animals.

Histologic analysis of intestinal epithelium, Peyer's patches and spleen's pulpa morphology after ŽP strain administration

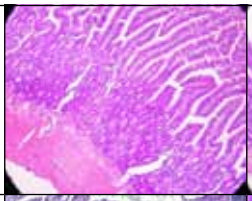
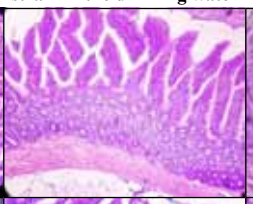
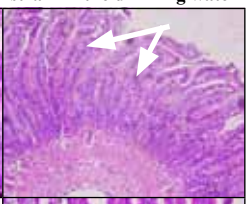
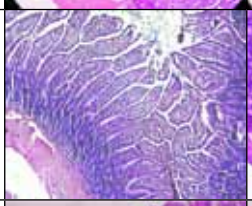
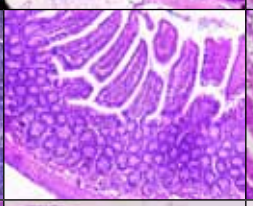
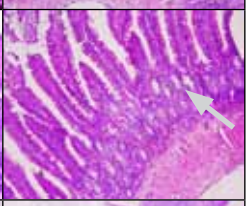
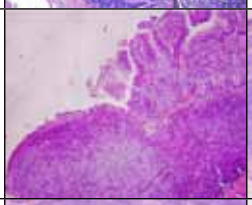
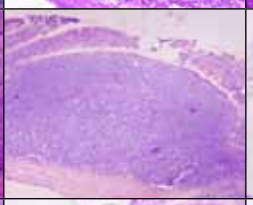
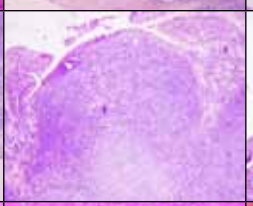
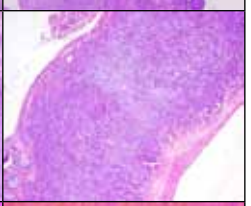
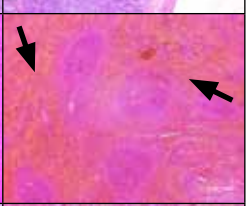
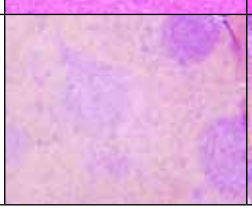
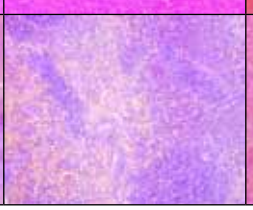
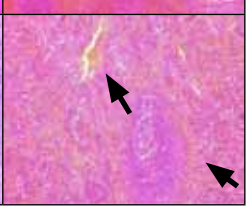
To verify the non-toxicity and non-pathogenicity of the ŽP strain for the host histological analysis of selected tissues was performed. Intestinal epithelium and Peyer's patches were chosen to confirm the non-toxicity and non-pathogenicity of ŽP strain for the intestine, the spleen was chosen to estimate the general immune reaction of the host to the ŽP strain.

As seen from Fig. 1, all studied organs in animals of both sexes had normal morphology in the control group. After administration of 5×10^8 CFU of ŽP, the structure of the small intestine, regardless of sex, was normal. The presence of large active lymphoid nodules, intraepithelial lymphocytic infiltration of the Peyer's patches in males and females indicated activation of lymphoid tissue. In the spleen moderate signs of stagnation of blood circulation together with activation of white pulp were found. These changes in spleen were greater in males than in females.

Table 1: Survival and weight of the animals.
Tabela 1: Preživetje in masa živali.

Characteristic	Control group	Administration of 5×10^8 CFU of the ŽP in the drinking water	Administration of 5×10^{10} CFU of the ŽP in the drinking water
Survival Day 21 (%)	100%	100%	100%
Average weight (g) of the animals - Day 1			
♂	175.25±10.31	175.25±10.31	175.25±10.31
♀	154.75±6.99	154.75±6.99	154.75±6.99
Average weight (g) of the animals - Day 21			
♂	221.80±24.52	228.00±17.18	259.25±15.78*
♀	187.00±20.80	207.80±8.37	192.00±16.05
Average daily gain (g)			
♂	2.21	2.51	4.00
♀	1.53	2.48	1.77
Mass increase compared to control group (%)			
♂	n.a.	2.80	16.88
♀	n.a.	10.65	2.67

n.a. not applicable; *significant difference from control group.

Tissue	Control	Administration of 5×10^8 CFU of the ŽP strain in the drinking water	Administration of 5×10^{10} CFU of the ŽP strain in the drinking water
Intestinal epithelium ♂ Magnification: 100×			
Intestinal epithelium ♀ Magnification: 100×			
Peyer's patches ♂ Magnification: 100×			
Peyer's patches ♀ Magnification: 100×			
Spleen's pulpa ♂ Magnification: 200×			
Spleen's pulpa ♀ Magnification: 200×			

Note: white arrows mark swelling of the small intestine mucosa; grey arrows mark swelling of the crypts of the small intestine; black arrows mark stagnation of blood in the red pulp of the spleen.

Figure 1: Histological examination of rat intestine, Peyer's patches and spleen after 21 days of the ŽP strain administration. Hematoxilyn-eosin staining.

Slika 1: Histološka analiza črevesja podgane, Peyerjevih ploščic in vranice po 21 dneh dodajanja seva ŽP. Barvanje hematoksilin-eozin.

Table 2: Biochemical parameters in blood serum after 21 days of the ŽP administration.
Tabela 2: Biokemijski parametri krvnega seruma po 21 dneh dodajanja seva ŽP.

Parameter	Control group	Administration of 5×10^8 CFU of the ŽP strain in the drinking water	Administration of 5×10^{10} CFU of the ŽP strain in the drinking water
Total protein (g/l)			
♂	63.16±0.74	64.44±1.90	65.78±0.97*
♀	69.20±1.02	65.90±0.70*	65.80±0.91*
Glucose (mmol/l)			
♂	9.80±0.84	9.96±0.84	11.15±1.18
♀	10.23±0.92	11.49±0.95	9.97±0.75
Total cholesterol (mmol/l)			
♂	1.48±0.05	1.52±0.06	1.51±0.08
♀	1.86±0.07	1.70±0.08	1.75±0.09
Calcium (mmol/l)			
♂	2.21±0.07	2.19±0.04	2.41±0.11
♀	2.37±0.12	2.24±0.02	2.21±0.07
Phosphorus (mmol/l)			
♂	2.60±0.08	2.89±0.13	2.54±0.19
♀	2.54±0.07	2.54±0.14	2.51±0.04
Creatinine (µmol/l)			
♂	49.92±2.26	45.86±4.24	52.63±2.23
♀	65.80±3.03	63.52±2.02	56.58±6.34
Urea (mmol/l)			
♂	6.07±0.42	6.47±0.36	7.44±0.19*
♀	8.61±0.22	7.82±0.39	8.94±0.32

*significant difference from control group.

After administration of 5×10^{10} CFU of the ŽP strain mucosal edema, diffuse leukocyte infiltration, and epithelial hypertrophy in the small intestine of male rats were observed, while vacuolization of epithelial cells, moderate expansion of the lumen of the crypts, activation of apical cells was found in females. Lymphoid tissue the Peyer's patches filled the entire mucosa of both sexes. Different sized lymphoid nodules fused to form a plateau. Stagnation of blood circulation was detected in the spleen. In the male group, stagnation in the stasis vessels was more pronounced than in the female group. To conclude the administration of 5×10^8 CFU of the ŽP strain was non-toxic and non-pathogenic for the host. However, the administration of 5×10^{10} CFU of ŽP strain had a toxic effect according to the structure changes of the studied organs.

The impact of per oral ŽP strain administration on biochemical parameters of blood

As seen from Table 2, the amount of total protein was significantly increased for the male rats in the group administered 5×10^{10} CFU of ŽP compared to the control group. On the contrary, the amount of total protein was decreased significantly compared to the control group in female rates in both experimental groups administered the ŽP strain. Further, the amount of urea was significantly different. The male group administered 5×10^{10} CFU of ŽP had a significantly increased amount of urea compared to the control, as well as the male group administered 5×10^8 CFU of ŽP. Among female rats, the amount of urea was increased in the group administered 5×10^{10} CFU of ŽP compared to the group administered 5×10^8 CFU of ŽP. For all the other analyzed characteristics, no significant differences were observed.

Based on these observations it can be assumed that a significant decrease in protein after the

administration of 5×10^{10} of ŽP for females may be a marker of active inflammation. An increase in urea in both groups (significant for males) may indicate an endogenous intoxication due to large doses of the ŽP strain.

The impact of per oral ŽP strain administration on hematological parameters of peripheral blood and microbicide activity of leukocytes and peritoneal macrophages

In the experimental group administered 5×10^8 CFU of ŽP there was no effect on the number of RBC and hemoglobin concentration in both female and male rats. However, in the experimental group administered 5×10^{10} CFU of ŽP a significant decrease in the number of RBC and hemoglobin concentration in female rats was observed. A decrease in the number of red blood cells after administration of ŽP strain in female, as well as the influence of sex hormones (Zaets et al. 2003, Pivkin et al. 2016) may explain less stagnation of blood cell circulation in female spleen. On the contrary, in males a significant increase in the number of RBC in this experimental group was revealed (Table 3). This is in accordance with the previous results of higher hemoglobin levels in male rats after probiotic administration (Salahuddin et al. 2013).

In the experimental group administered 5×10^8 CFU of the ŽP there was no effect on the absolute number of different population of leukocytes in peripheral blood in both female and male rats.

Table 3: Analysis of the red blood cell, total leukocyte count in peripheral blood after 21 days of the ŽP strain administration.

Tabela 3: Analiza rdečih krvniček, števila levkociotv v periferni krvi po 21 dneh dodajanja seve ŽP.

Parameter	Control group	Administration of 5×10^8 CFU of the ŽP in the drinking water	Administration of 5×10^{10} CFU of the ŽP in the drinking water
RBC (10^6/mm^3)			
♂	4.52±0.06	4.66±0.11	4.88±0.23*
♀	4.74±0.11	4.46±0.18	4.16±0.13*
HGB (g/dl)			
♂	14.48±0.22	14.54±0.43	14.28±0.76
♀	15.26±0.45	14.78±0.58	13.84±0.55*
Le (10^3/mm^3)			
♂	10.70±1.72	8.16±3.04	7.20±2.35
♀	6.78±2.26	8.16±3.05	10.70±1.72*
Lym (10^3/mm^3)			
♂	8.16±1.09	6.41±2.31	5.29±1.71*
♀	5.68±2.25	6.66±2.62	9.54±1.38*
Neu (10^3/mm^3)			
♂	2.12±0.77	1.29±0.49	1.39±0.48
♀	0.68±0.30	0.97±0.36	0.66±0.27
Mon (10^3/mm^3)			
♂	0.28±0.12	0.33±0.25	0.26±0.029
♀	0.12±0.04	0.22±0.13	0.12±0.03
Eos (10^3/mm^3)			
♂	0.14±0.05	0.08±0.06	0.18±0.13
♀	0.23±0.09	0.25±0.10	0.33±0.10
Immature Neu (10^3/mm^3)			
♂	0.10±0.01	0.42±0.06	0.06±0.03*
♀	0.30±0.03	0.40±0.05	0.20±0.02

*significant difference from control group.

There was a significant increase of the absolute number of leukocytes and the absolute number of lymphocytes in the female rats in the experimental group administered 5×10^{10} of the ŽP strain.

On the other hand, for male rats in the experimental group administered 5×10^{10} of ŽP only the absolute number of lymphocytes changed, it namely significantly decreased (Table 3).

Further, significant decrease in the number of immature neutrophils was observed among male rats in the experimental group administered 5×10^{10} of ŽP. Based on this observation it can be concluded that the administration of 5×10^{10} of ŽP enhanced the neutrophils maturation process. For all the other studied parameters no differences between experimental groups and the control group were observed.

As seen from Table 4, in the experimental group administered 5×10^{8} CFU of ŽP there was no effect on the microbicide activity of the leukocytes and peritoneal macrophages of rats of both sexes. A significant decrease in microbial killing activity of leukocyte suspension was observed among the male rats in the experimental group administered 5×10^{10} CFU of ŽP. Given that the number of phagocytes (Table 3) in the peripheral blood did not change, it can be assumed that 5×10^{10} CFU of ŽP in the diet may reduce the microbicidal activity of peripheral blood cells. Administration of the ŽP strain did not affect microbial killing of leukocytes in female rats and macrophages in both sexes.

Further, it was found that the spontaneous level of ROS production by leukocyte suspension was reduced only among female rats in the experimental group administered 5×10^{10} CFU of ŽP (Table 4). That is due to a decrease of functional activity of phagocytic cells was revealed (Table 3). This means, despite the increase in the absolute number of leukocytes in female, the functional activity of peripheral blood phagocytes decreases under the influence of excessive concentration of probiotic. Females are more sensitive to the effects of excessive amounts of probiotic.

Table 4: *Ex vivo* analysis of functional activity of leukocytes of peripheral blood and peritoneal macrophages after 21 days of the ŽP strain administration.

Tabela 4: Analiza funkcionalne aktivnosti levkocitov iz periferne krvi in peritonealnih makrofagov *ex vivo* po 21 dneh dodajanja seva ŽP.

Parameter	**Control group**	**Administration of 5×10^{8} CFU of the ŽP in the drinking water**	**Administration of 5×10^{10} CFU of the ŽP in the drinking water**
Microbial killing by leukocytes (%)			
♂	88.0±1.1	85.6±0.9	79.3±3.8*
♀	83.4 ±1.8	84.4 ±0.9	80.7 ±1.1
Microbial killing by macrophage (%)			
♂	74.7±3.5	76.7±3.5	51.6±11.3
♀	68.2 ±5.0	71.2 ±5.1	81.4 ±1.0
Spontaneous ROS production by leukocytes (% of control)			
♂	#	82.5±4.9	152.5±77.2
♀		103.1±4.0	70.3±4.7*
Stimulated ROS production by leukocytes (% of control)			
♂	-	80.4±14.2	108.8±27.4
♀		105.6±19.5	159.7±43.9
Spontaneous ROS production by macrophage (% of control)			
♂	-	114.8±1.9	106.8±21.7
♀		102.2±50.0	184.7±62.0
Stimulated ROS production by macrophage (% of control)			
♂	-	93.4±11.7	233.9±110.51
♀		168.2±98.9	316.0±127.6

*significant difference from control group; # control group ROS production is taken as 100%.

There was no significant stimulating effect on spontaneous and stimulated ROS production on peritoneal macrophages in male and female rats. But according to other study the main activation mechanism of probiotics on immune system is through presence of probiotic particles inside macrophages, that induce expression on its surface TLR2 and mannose receptors (Maldonado et al. 2019).

Conclusions

Probiotics had been used as growth promoters due to their ability to suppress the growth and activities of growth depressing microbiota and their ability of enhancing absorption of nutrients through the production of digestive enzymes (Ben Lagha et al. 2017). The probiotic *E. coli* strain Nissle 1917 enhances barrier function of human intestinal epithelial cells against different enteroinvasive bacterial pathogen. Further, it induces the development of the gut immune system in animal models and human newborns (Sonnenborn 2009). Our results showed that the ŽP strain, which is based on the *E. coli* Nissle 1917 strain, when administered at 5×10^8 CFU in the drinking water is safe and responsible for maintenance of an adequate non-specific resistance and can also improve the performance of animals in terms of weight gain. Hence our results indicate that it is a promising probiotic.

Povzetek

Leta 2014 je bilo objavljeno poročilo z oceno, da bo do leta 2050 na svetu 10 milijonov smrtnih primerov zaradi protimikrobne odpornosti bakterij. WHO je tako leta 2017 izdala seznam prednostnih bakterijskih patogenov za raziskave in razvoj novih protimikrobnih zdravil. Starčič Erjavec in sod. so razvili sev ŽP, nov antimikrobni sev na osnovi seva Nissle 1917, ki vsebuje na konjugaciji temelječ protimikrobni dejavnik, ki ga sestavljata gen "kill" (gen za bakteriocin, kolicin ColE7) in gen "anti-kill" (gen za imunost proti kolicinu E7). V prejšnjih raziskavah je bila učinkovitost tega na konjugaciji temelječega protimikrobnega sistema dokazana že na različnih sevih bakterije *Escherichia coli*. V tej študiji smo preučevali učinek tega seva po peroralni uporabi na gostitelja, in sicer na podgane obeh spolov. Uporabljali smo podgane linije Wistar, stare 4 tedne. Vse delo s podganami je bilo odobreno s strani etične komisije Permske državne medicinske univerze.

Živali, razdeljene v skupini samcev in samic, so bile dalje razdeljene o v naslednje eksperimentalne skupine: 1 - kontrolna skupina, ki je prejemala hrano in sterilno pitno vodo v 400-mililitrski steklenici; 2 - poskusna skupina, ki je prejela hrano + 5×10^8 CFU seva ŽP v 400-mililitrski steklenici pitne vode; 3 - poskusna skupina, ki je prejela hrano + 5×10^{10} CFU seva ŽP v 400-mililitrski steklenici pitne vode. Vse živali so med poskusom dobivale hrano v enakih količinah. Gojili smo jih v kletkah s po 5 živalmi, v prostoru z rednim 12h dnevno-nočnim ciklom pri temperaturi 20±2 °C. Poskus je potekal 21 dni, na kar smo izvedli analizo. Parametri, ki smo jih preučevali so bili masa živali, histologija črevesja, vranice in Peyerjevih plošic, biokemija krvi, hematologija in mikrobicidna aktivnost levkocitov in makrofagov.

V primerjavi s kontrolno skupino se je povprečna teža (g) pri obeh poskusnih skupinah živali obeh spolov, ki so prejemale sev ŽP v pitni vodi, povečala za vsaj 2%. Histološka analiza črevesnega epitelija, Peyerjevih ploščic in morfologije vranice je pokazala, da je dodajanje 5×10^8 CFU seva ŽP bilo za podgane obeh spolov netoksično in nepatogeno. Dodajanje 5×10^{10} CFU seva ŽP pa je vplivalo toksično, saj smo opazili strukturne spremembe preučevanih tkiv. Biokemijska analiza krvi je razkrila, da se je pri samicah značilno zmanjšala količina proteinov v krvi po dodajanju 5×10^{10} seva ŽP, kar je lahko bila posledica vnetja. Pri tej količini seva ŽP se je pri obeh spolih povečala količina uree v krvi, kar je verjetno bila posledica toksičnega učinka te doze seva ŽP. Nadalje je hematološka analiza pokazala, da je pri samicah, ki smo jim dodajali 5×10^{10} seva ŽP prišlo do značilnega zmanjšanja števila eritrocitov in količine hemoglobina. Nasprotno je pri samcih, ki smo jim dodajali 5×10^{10} seva ŽP, prišlo do povečanja števila eritrocitov. Pri samicah, ki smo jim dodajali 5×10^{10} seva ŽP je prišlo do značilnega povišanja tako števila levkocitov, kot števila limfocitov in odstotka limfocitov, med tem ko je pri samcih v tej skupini prišlo do značilnega

znižanja števila limfocitov. Analiza funkcionalne aktivnosti levkocitov iz periferne krvi *ex vivo* je pokazala, da je pri samicah, ki smo jim dodajali 5×10^{10} seva ŽP prišlo do značilnega zmanjšanja produkcije ROS. Naši rezultati so pokazali, da je sev ŽP, če ga dajemo v odmerku 5×10^{8} CFU v pitni vodi, varen in odgovoren za nadzor in vzdrževanje ustreznega lokalnega in nespecifičnega imunskega odzivaima ter lahko tudi izboljša status podgane v smislu povečanja telesne mase. Zato naši rezultati kažejo, da gre za obetaven probiotik.

Acknowledgments

The study was carried out in the framework of the scientific project No.C-26/792 supported financially by the Government of Perm Krai.

References

Aboderin, F.I., Oyetayo, V.O., 2006. Haematological studies of rats fed different doses of probiotic, *Lactobacillus plantarum*, isolated from fermenting corn slurry. Pak. J. Nutrition, 5 (2), 102–105.

Albiger, B., Glasner, C., Struelens, M., Grundmann, H., Monnet, D.L., 2015. European Survey of Carbapenemase–Producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill, 20 (45).

Allocati, N., Michele, M., Alexeyev, M.F., Ilio, D.C., 2013. *Escherichia coli* in Europe: An Overview. Int. J. Envir. Res. Pub. Health, 10, 6235–6254.

Arribas, B., Rodríguez-Cabezas, M.E., Camuesco, D., Comalada, M., Bailón, E., Utrilla, P., Nieto, A., Concha, A., Zarzuelo, A., Gálvez, J., 2009. A probiotic strain of *Escherichia coli*, Nissle 1917, given orally exerts local and systemic anti-inflammatory effects in lipopolysaccharide-induced sepsis in mice. Br. J. Pharmacol., 157 (6), 1024–1033.

Ben Lagha, A., Haas, B., Gottschalk, M., Grenier, D., 2017. Antimicrobial potential of bacteriocins in poultry and swine production. Vet. Res., 48, 22.

Boulianne, M., Arsenault, J., Daignault, D., Archambault, M., Letellier, A., Dutil, L., 2016. Drug use and antimicrobial resistance among *Escherichia coli* and *Enterococcus* spp. isolates from chicken and turkey flocks slaughtered in Quebec, Canada. Can. J. Vet. Res., 80 (1), 49–59.

Davis, G.S., Waits, K., Nordstrom, L., Grande, H., Weaver, B., Papp, K., Horwinski, J., Koch, B., Hungate, B.A., Liu, C.M., Price, L.B., 2018. Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. BMC Microbiol., 18 (1), 174.

Ewers, C., Bethe, A., Semmler, T., Guenther, S., Wieler, L.H., 2012. Extended-spectrum beta-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: A global perspective. Clin. Microbiol. Infect., 18, 646–655.

GOST 34088-2017. Rukovodstvo po soderzhaniyu i uhodu za laboratornymi zhivotnymi. Pravila soderzhaniya i uhoda za sel'skohozyajstvennymi zhivotnymi/GOST 34088-2017. Guide for the maintenance and care of laboratory animals. Rules for the maintenance and care of farm animals.

Karlowsky, J.A., Lob, S.H., Kazmierczak, K.M., Bada, R.E., Young, K., Motyl, M.R., Sahm, D.F., 2017. In vitro activity of imipenem against carbapenemasepositive Enterobacteriaceae isolates collected by the SMART Global Surveillance Program from 2008 to 2014. J. Clin. Microbiol., 55, 1638–1649.

Koga, V.L., Rodrigues, G.R., Scandorieiro, S., 2015. Evaluation of the antibiotic resistance and virulence of *Escherichia coli* strains isolated from chicken carcasses in 2007 and 2013 from Parana, Brazil. Foodborne Pathog. Dis., 12 (6), 479–485.

Maldonado, G.C., Cazorla, S.I., Lemme Dumit, J.M., Vélez, E., Perdigón, G., 2019. Beneficial effects of probiotic consumption on the immune system. Ann. Nutr. Metab., 74 (2), 115–124.

Maslennikova, I.L., Kuznetsova, M.V., Nekrasova, I.V., Shirshev, S.V., 2017. Effect of bacterial components of mixed culture supernatants of planktonic and biofilm Pseudomonas aeruginosa with commensal Escherichia coli on the neutrophil response in vitro. Pathog. Dis., 75 (8), 1-12.

Maslennikova, I.L., Kuznetsova, M.V., Toplak, N., Nekrasova, I.V., Zgur Bertok, D., Starcic Erjavec, M., 2018. Estimation of the bacteriocin ColE7 conjugation-based "kill"-"anti-kill" antimicrobial system by real-time PCR, fluorescence staining and bioluminescence assays. Lett. Appl. Microbiol., 67 (1), 47–53.

Mountzouris, K.C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G., Fegeros K., 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus, Bifidobacterium, Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating fecal microflora composition and metabolic activities. Poultry Sci., 86 (2), 309–317.

Mountzouris, K.C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., Fegeros, K., 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and fecal microflora composition. Poultry Sci., 89, 58–67.

O'Neill, J., 2014. Review on Antimicrobial Resistance Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. London: Review on Antimicrobial Resistance. Available from: https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

Pivkin, I.V., Peng, Z., Karniadakis, G.E., Buffet, P.A., Dao, M., Suresh S., 2016. Biomechanics of red blood cells in human spleen and consequences for physiology and disease. Proc. Natl. Acad. Sci. USA, 113 (28), 7804–7809.

Salahuddin, M., Akhter, H., Akte,r S., Miah, M.A., Ahmad, N., 2013. Effects of probiotics on haematology and biochemical parameters in mice. Bangladesh Vet., 30, 20–24.

Silva, V.O., Foureaux, R.C., Araujo, T.S., Peconick, A.P., Zangeronimo, M.G., Pereira, L.J., 2012. Effect of probiotic administration on the immune response: a systematic review of experimental models in rats. Braz. Arch. Biol. Technol., 55, 685–694.

Sonnenborn, U., 2009. The non-pathogenic Escherichia coli strain Nissle 1917-features of a versatile probiotic. Microb. Ecol. Health Disease, 21 (3), 122–158.

Starčič Erjavec, M., Petkovšek, Ž., Kuznetsova, M.V., Maslennikova, I.L., Žgur-Bertok, D., 2015. Strain ŽP - the first bacterial conjugation-based "kill"-"anti-kill" antimicrobial system. Plasmid, 3 (82), 28–34.

Timmerman, H.M., Veldman, A., Van den Elsen, E., Rombouts, F.M. and Beynen, A.C., 2006. Mortality and growth performance of broilers given drinking water supplemented with chickenspecific probiotics. Poultry Sci., 85, 1383–1388.

Van Duin, D., Doi, Y., 2017. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence, 8, 460–469.

Wehkamp, J., Harder, J., Wehkamp, K., Wehkamp-von Meissner, B., Schlee, M., Enders, C., Sonnenborn, U., Nuding, S., Bengmark, S., Fellermann, K., Schröder, J.M., Stange E.F., 2004. NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. Infect. Immun., 72 (10), 5750–5758.

WHO, 2017. World Health Organization. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. Geneva: WHO Press, pp. 1–7.

Zaets, S.B., Berezina, T.L., Xu, D.Z., Lu, Q., Ricci, J., Cohen, D., Ananthakrishnan, P., Deitch, E.A., Machiedo, G.W., 2003. Burn-induced red blood cell deformability and shape changes are modulated by sex hormones. Am. J. Surg., 186 (5), 540–546.