Polish Journal of Veterinary Sciences Vol. 22, No. 1 (2019), 5–10

DOI 10.24425/pjvs.2018.125616

Original article

Evaluation of *Lactobacillus* spp. and yeast based probiotic (Lavipan) supplementation for the reduction of *Salmonella* Enteritidis after infection of broiler chickens

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Abstract

The number of human cases of salmonellosis in the EU was 94,625 in 2015. Considering the source of these infections, *Salmonella* spp. was most frequently detected in broiler chicken meat and *Salmonella* Enteritidis (SE) was the most commonly reported serovar.

The efficacy of probiotics in limiting *Salmonella* spp. infection in poultry has been demonstrated in numerous papers. The administration of probiotics at the level of primary production reduces the risk of contamination of poultry food products with *Salmonella* spp.

A study was carried out in order to determine the potential for reducing the *Salmonella* spp. population in broiler chickens with the use of the Lavipan (JHJ, Poland) probiotic that comprised selected stains of lactic acid bacteria and *Saccharomyces cervisae*.

Salmonella spp.-free broiler chickens were divided into two groups and received the same feed with (group L) or without (group C) the probiotic throughout the experiment. All day-old chickens were infected *per os* with SE. Samples of cecum content were collected 2, 4, and 6 weeks after SE infection and pectoral muscles were collected 6 weeks following SE infection for the evaluation of the SE population number. Serum samples for serological examinations were collected 6 weeks after infection.

Six weeks after infection, the number of SE-positive cecal samples was lower in the L group (12.5% positive) in comparison to the C group (87.5%). Similar results were demonstrated for the muscle samples (25% in contrast to 87.5%). At the same time, in both cases, the SE CFU/g was significantly lower in the L group. The results of our study indicate that Lavipan was capable of reducing the population of SE in the gastrointestinal tract, which eventually improved the hygienic parameters of the pectoral muscles.

Four weeks after infection, SE was not detected in any of the experimental groups. In both groups, no specific anti-SE antibodies were detected.

Key words: chicken, probiotic, *Salmonella* Enteritidis, serological response

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Introduction

Non-typhoidal *Salmonella* is one of the major causes of foodborne gastrointestinal infections in humans worldwide. As demonstrated by the European Food Safety Authority (EFSA), the number of human cases of salmonellosis was 94,625 in 2015, as reported by 28 EU Member States (EFSA 2016). Contaminated eggs and poultry meat are considered the major sources of *Salmonella* spp. for humans (Osimani et al. 2016). In 2015, *Salmonella* spp. was most frequently detected in broiler chicken meat (EFSA 2016). The two most commonly reported serovars were *Salmonella* Enteritidis (SE) and *S.* Typhimurium (ST), representing 45.7% and 15.8% respectively, of all reported serovars in human salmonellosis (EFSA 2016).

Strategies aimed at minimizing the risk of infection in commercial poultry with *Salmonella* spp. can be roughly divided into two action pathways. The first pathway involves obtaining biological material free of infection with these bacteria (including the official monitoring of reproductive poultry flocks during the rearing and laying period and/or immunoprophylaxis), while the second pathway consists of biosecurity (Cox and Pavic 2010). Despite this and monitoring surveys carried out for many years, and the elimination of *Salmonella* spp. infections in the poultry population, infections with these bacteria still pose a significant problem in commercial poultry production (EFSA 2016, Zebrowska et al. 2017). The issue of *Salmonella* spp. infections results not only from the epidemiology of such infections in humans, but also from the consequences of their presence in poultry, which include the need for the sanitary slaughter of a flock or the withdrawal of products derived from such birds from the market and their utilization. Detection of flagellated *Salmonella* spp. bacteria in food has a very negative impact on the potential export of such foodstuffs.

For many years, probiotic products have been considered one of the alternative methods for limiting infections and contamination with *Salmonella* spp. in poultry. The phenomenon of competitive exclusion (CE), understood as the ability of probiotic microorganisms to prevent the pathogenic organism from colonizing the gastrointestinal tract, is one of the crucial elements underlying the beneficial effects of probiotics (Smith 2014, Forkrus et al. 2017). The efficacy of probiotics in limiting the capability of S*almonella* spp. for invasiveness in poultry has been demonstrated in numerous papers and for different multi-species or single strain probiotics (Pascual et al. 1999, Avila et al. 2006, Higgins et al. 2008, Carter et al. 2017, Oh et al. 2017). Probiotics, therefore, can be considered as a third action in the network for minimizing the risk of developing active *Salmonella* spp. infections in commercial poultry.

Considering the above, a study was carried out to determine the potential for reducing the *Salmonella* spp. population in the cecal content and in the pectoral muscles of broiler chickens, inoculated *per os* with *Salmonella* Enteritidis, by supplementing the feed with the Lavipan (JHJ, Poland) probiotic.

Materials and Methods

The experimental and animal handling procedures were conducted with the approval of the Local Ethics Committee for Animal Experiments in Olsztyn, Poland (approval number: 49/2017).

Experiment design

The experiment was carried out with 48 Ross-308 broiler chickens hatched at a commercial hatchery. The chicks were not vaccinated against any disease. At the start of the experiment, cloaca swabs from 10 chicks were collected to identify the presence of *Salmonella* spp. Afterwards, the birds were randomly divided into 2 groups of 24 animals each: control (C) and experimental (L). Throughout the trial, the L birds were administered Lavipan at a dose of 0.5 kg (producer recommendation) per 999.5 kg of feed, whereas the C birds were fed the same feed without the probiotic. Water and feed were provided *ad libitum* throughout the experimental period. The feed type (from a "starter", then "grower" and finally "finisher") was changed the day before the samples for microbiological assays were collected. The complete feed was provided by Tasomix (Poland).

Twelve hours after the first feed was administered, the birds were inoculated *per os* with the S16/1321 *S.* Enteritidis (SE) strain at a dose of 7.5×10^3 CFU in 0.25 mL (PBS)/bird. The SE strain was kindly provided by Prof. Dariusz Wasyl of the National Veterinary Research Institute, Puławy, Poland.

On 14, 28 and 42 days of life, eight birds from each group were euthanized and the cecum content was then sampled for microbiological evaluation in order to count the SE population. In addition, on day 42, samples of deep pectoral muscles were collected. The type of samples collected and the time of sampling are summarized in Table 1.

The samples of cecum content (and from the pectoral muscles) were collected with sterile surgical instruments, using one set of tools per sample. The skin at the sampling site was disinfected with 40% alcohol. The cecum was exposed and cut at the ileal-cecal junction, incised along the wall, and its content was collected from the mucosa into sterile 50-mL Falcon-type tubes.

Table 1. Experimental layout summarized with the character and number of samples collected for microbiological examination.

Sampling number - bird age	Samples collected (from number (n) of birds in each group)	
	Caecum content	Deep pectoral muscles
I - 14 dol[1]	+[2] (n=8)	-[3]
II - 28 dol	+ (n=8)	-
III - 42dol	+ (n=8)	+ (n=8)

[1] dol - day of life
[2] "+" indicates that these samples were collected from birds during sampling
[3] "-" indicates that these samples were not collected from birds during sampling

The pectoral muscle samples were taken from the left deep pectoral muscle, having cut the disinfected skin and underlying superficial pectoral muscles. Before the muscles were subjected to microbiological evaluation each muscle sample was individually homogenized.

On day 42, blood samples for serological evaluation were collected from eight birds from each group to determine the titre of specific anti-SE antibodies using the ELISA.

The trial was conducted in isolated pens of the Laboratory of Experimental Poultry Infections, at the Department of Avian Diseases, University of Warmia and Mazury in Olsztyn.

SE preparation for experimental infection

In order to prepare the suspension for the experimental infection, the S16/1321 SE strain was transferred to a Columbia agar (Oxoid, UK) and incubated at 40.5°C for 24 hours. After incubation, the SE was suspended in 0.85% sterile PBS (Sigma-Aldrich, Germany). With the use of a spectrophotometer (DENSI-LA-METER II, Erba Lachema, Czech Republic). the final concentration of the SE suspension (7.5×10^3 CFU/0.25 ml) was prepared and used for the infection of broiler chickens within one hour.

In order to evaluate the actual CFU in the infection dose, tenfold dilutions of the SE suspension were prepared and placed (1 mL, in duplicate) on plates with a chromogenic medium (Brilliance Salmonella, Oxoid, UK). The plates were incubated at 40.5°C for 24 h. Once incubated, the colonies were counted and CFU was determined.

Probiotic

Group L received in feed the Lavipan probiotic product (JHJ, Poland), which comprises selected stains of lactic acid bacteria: *Lactococcus lactis* IBB 500 (origin – chicken feces), *Carnobacterium divergens* S-1 (origin – carp gut), *Lactobacillus casei* ŁOCK 0915 (origin – chicken feces) and *Lactobacillus plantarum* ŁOCK 0862 (origin – turkey feces) at 1×10^9 CFU/g each, and *Saccharomyces cervisae* ŁOCK 0141 (origin – plant silage) at 1×10^7 CFU/g.

Microbiological examination

The samples' initial suspension and tenfold dilutions were prepared according to the PN-EN ISO 6887-1 and PN-EN ISO 6887-2 standards.

From each dilution, 1 mL was taken with a sterile pipette and placed onto plates with a chromogenic medium (Brilliance Salmonella, Oxoid, UK), and then evenly distributed with a sterile spreader. The plates were incubated at 40.5°C for 24 h.

Once incubated, the colonies were counted and CFU/g was determined for the investigated samples.

Typical colonies were selected from each chromogenic medium plate and then transferred to the Columbia agar (Oxoid, UK) in order to identify the taxonomy of *Salmonella* colonies and classify them as the SE serovar. The plates were incubated at 40.5°C for 24 h. The identification of *Salmonella* spp. was performed in accordance with the PN-EN ISO 6579 standard. SE serotyping was carried out with a Salmonella Big Five Serotyping Kit (SSI Diagnostica, Denmark).

Serological examination

Serological evaluation was performed with a commercial ELISA SE antibody test kit (IDEXX, USA) according to the manufacturer's recommendations. The ELISA test was carried out with the use of an Eppendorf epMotion 5075 LH automated pipetting station (Eppendorf, Germany), a BioTek EL × 405 automatic plate washer (BioTek, USA) and a BioTek EL × 800 plate reader. The sample-to-positive (S/P) ratio was calculated based on the ODs and used to express the mean (S/P)-ratio +/– SD per group.

Table 2. Microbiological results summarized. Results are presented as the number of samples positive/tested (P/T) and mean *Salmonella* Enteritidis (SE) CFU/g +/– SD. Statistical analysis was performed with the differences in mean SE CFU/g between C and L groups in each sampling.

Group	Sampling I		Sampling II		Sampling III			
	Cecum content		Cecum content		Cecum content		Pectoral muscles	
	P/T	CFU/g +/- SD	P/T	CFU/g +/- SD	P/T	CFU/g +/- SD	P/T	CFU/g +/- SD
C	8/8	101.9 +/- 98.9 × 10^3	0/8	0.0	7/8	42.5 +/- 42.1 × 10^4	7/8	5 +/- 3.2 × 10^1
L	8/8	64.8 +/- 30.4 × 10^3	0/8	0.0	1/8	0.75 +/- 2.1 × 10^{3*}	2/8	1.5* +/- 2.8 × 10^{1*}

* Significant differences (Student t-test, * as $p<0.05$) compared to the control group (C).

Table 3. Serological results summarized. Results are presented as the number of samples positive/tested (P/T) with mean sample/positive (S/P) ratio +/– SD.

Group	P/T	Mean S/P +/- SD
C	0/8	0.017 +/- 0.022
L	0/8	0.022 +/- 0.022

Statistical analysis

The results were processed statistically with the Student t-test and with Graph-Pad Prism 6.0 software. The differences were considered statistically significant at $p<0.05$.

Results

Microbiology

At the beginning of the experiment, the chicks were free from *Salmonella* spp. (data not shown). The actual concentration of SE suspension used for infection was at exactly 7.5 × 10^3 CFU/0.25 mL.

The results of the microbiological analysis are summarized in Table 2. Two weeks after the infection all of the cecal samples in both L and C groups were positive for SE. Mean SE CFU/g accounted for 64.8 x 10^3 and 101.9 x 10^3 in L and C groups, respectively.

None of the cecal samples were positive for SE growth in either L or C group during the second sampling.

During the third microbiological investigation, significant differences were found in the mean SE CFU/g between the C (42.5 × 10^4 CFU/g) and L (1.5 × 10^1 CFU/g) groups ($p = 0.013$). The growth of SE in the L group was detected in 1 out of the 8 examined cecal samples, while in the C group 7 out of the 8 investigated samples were positive. For each positive sample in the C group, the SE CFU/g was one logarithmic level higher than that of the single positive sample from the L group.

Six weeks after infection SE was detected in 2 of the 8 examined pectoral muscle samples collected from the L group, whereas it was detected in 7 of the 8 samples from the C group. At the same time, mean SE CFU/g in pectoral muscles of the L group (1.5 x 10^1 CFU/g) was significantly lower ($p = 0.035$) than in the C group (5 × 10^1 CFU/g).

Serology

The results of serological evaluation of anti-SE antibodies 6 weeks after experimental infection are summarized in Table 3. No anti-SE specific antibodies were detected in any of the groups.

Discussion

The phenomenon by which the normal intestinal microflora protects the host against invading pathogens is called competitive exclusion (Schneitz 2005). CE, provided by the gut natural microflora or probiotics is considered to result from a range of direct (i.a. production of volatile fatty acids and competition for colonization site) and indirect (i.a. immune system stimulation) effects (Doyle and Erickson 2006). Therefore, probiotic application in poultry is increasingly considered as an alternative solution, allowing a significant degree of reduction of infections with microorganisms potentially pathogenic to humans. For the two types of pathogens that are the most common cause of foodborne diseases in humans, i.e. *Campylobacter* spp. and *Salmonella* spp., numerous studies have been conducted with the results

indicating the beneficial effects of probiotics associated with limiting the population of these pathogens in poultry (Pascual et al. 1999, Avila et al. 2006, Higgins et al. 2008, Baffoni et al. 2017, Carter et al. 2017, Mañes-Lázaro et al. 2017, Oh et al. 2017, Saint-Cyr et al. 2017). Probiotics are thus commonly believed to be a very beneficial tool for improving the sanitary parameters of poultry food products through their implementation at the primary stage of poultry production.

Additionally, numerous studies with the use of commercially available (at the time of the experiment) probiotics indicate that these products can successfully protect chickens against Salmonella spp. infection under both laboratory and field conditions (Bolder et al. 1992, Cameron and Carter 1992, Nuotio et al. 1992, Wierup et al. 1992, Methner et al. 1997, Palmu and Camelin 1997, Stern et al. 2001, Schneitz 2005).

The inhibitory effects of Lactic acid bacteria and yeasts that comprise the probiotic used in this study (Lavipan) on *S.* Enteritidis and *S.* Typhimurium growth *in vitro* has been evaluated previously and those probiotic strains revealed a very promising potential to reduce the growth of pathogenic bacteria (Burchardt, personal communication, 2015). Additionally Lavipan probiotic has been shown to be effective in reducing *Campylobacter* spp. population in broiler chickens in a study performed under field conditions and it has been shown to possess immunomodulatory properties (Smialek et al. 2018). In the current experiment, we investigated the effects of this multi-species probiotic on the population of *S.* Enteritidis in the cecum and pectoral muscles of broiler chickens after SE experimental infection of day-old birds.

In the present study the Lavipan probiotic significantly reduced the growth of the SE population in the cecal content of broiler chickens 6 weeks after the experimental infection on the first day of the birds' life. Additionally, the data indicate that two weeks after infection, the SE CFU/g in the cecal content of the birds that received the probiotic was lower than in the control group, which suggests a different dynamic of growth for the pathogenic microorganisms in the digestive tract of the birds fed a diet supplemented with Lavipan. The results of our study are consistent with previous findings that *Lactobacillus* spp. based probiotics are capable of reducing SE infection in broiler chickens (Higgins et al. 2008, Viscente et al. 2008). Additionally, Shibat El-hamd and Mohamed (2016) demonstrated that *L.* acidophilus, *L.* plantarum and *S. cerevisiae* based probiotic was capable of successively reducing the number of SE population in the cecal content of experimentally infected broiler chickens.

Based on the results of the present study, it can be concluded that the investigated Lavipan probiotic, administered at 0.5 kg per 999.5 kg of feed, reduces the risk of active *Salmonella* Enteritidis infection in broiler chickens. The study demonstrated its efficacy in creating the conditions allowing for the competitive exclusion of SE in the gastrointestinal tract, contributing to the reduction of the risk of pectoral muscle infection with these bacteria.

It is difficult to determine why, during the second sampling (day 28 of life), SE was not detected in any of the groups. We assume that at this time after infection, a certain form of "homoeostasis" developed between the SE and the host which made detection of SE in the cecal content impossible.

The absence of anti-SE specific antibodies 6 weeks after the infection probably resulted from the low infectious dose of SE used for the experimental infection; this has already been demonstrated for experimental infection with low SE doses (Velhner et al. 2005). The SE dose used in our study ensured a subclinical course of infection (with cecum and pectoral muscle colonization) without the involvement of the systemic immune mechanisms. Conversely, the possibility that the lack of humoral immunity stimulation resulted from the low immunogenicity of the SE strain used in the study cannot be excluded.

Conclusions

It has been demonstrated previously that probiotics inhibit the growth of pathogenic microflora in the gastrointestinal tract through competitive exclusion. The results of our study allow for the conclusion that the Lavipan probiotic (JHJ, Poland), comprising selected stains of lactic acid bacteria and *Saccharomyces cervisae* yeasts, added to the feed mixture for broiler chickens, was capable of reducing the population of SE in their cecum, which eventually contributed to the improved hygienic parameters of their pectoral muscles. Considering the alarming yearly EFSA reports concerning the number of human cases of Salmonellosis in the EU, it is worth emphasizing that besides vaccination and biosecurity measures, implementation of probiotics at different stages of poultry production could reduce the risk of contamination of poultry products with *Salmonella* spp.

Acknowledgements

This publication was supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of the Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

References

Avila LA, Nascimento VP, Salle CT, Moraes HL **(2006)** Effects of probiotics and maternal vaccination on Salmonella enteritidis infection in broiler chicks. Avian Dis 50: 608-612.

Baffoni L, Gaggia F, Garofolo G, Di Serafino G, Buglione E, Di Giannatale E, Di Gioia D **(2017)** Evidence of Campylobacter jejuni reduction in broilers with early synbiotic administration. Int J Food Microbiol 251: 41-47.

Bolder NM, van Lith LA, Putirulan FF, Jacobs-Reitsma WF, Mulder RW **(1992)** Prevention of colonization by *Salmonella enteritidis* PT4 in broiler chickens. Int J Food Microbiol 15: 313-317.

Cameron DM, Carter JN **(1992)** Evaluation of the efficacy of Broilact in preventing infection of broiler chicks with *Salmonella enteritidis* PT4. Int J Food Microbiol 15: 319-326.

Carter A, Adams M, La Ragione RM, Woodward MJ **(2017)** Colonisation of poultry by Salmonella Enteritidis S1400 is reduced by combined administration of Lactobacillus salivarius 59 and Enterococcus faecium PXN-33. Vet Microbiol 199: 100-107.

Cox JM, Pavic A **(2010)** Advances in enteropathogen control in poultry production. J Appl Microbiol 108: 745-755.

Doyle MP, Erickson MC **(2006)** Reducing the carriage of foodborne pathogens in livestock and poultry. Poult Sci 85: 960–973.

EFSA **(2016)** The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2015. EFSA J 14: 4634.

Forkus B, Ritter S, Vlysidis M, Geldart K, Kaznessis YN **(2017)** Antimicrobial Probiotics Reduce Salmonella enterica in Turkey Gastrointestinal Tracts. Sci Rep 7: 40695.

Higgins SE, Higgins JP, Wolfenden AD, Henderson SN, Torres-Rodriguez A, Tellez G, Hargis B **(2008)** Evaluation of a Lactobacillus-based probiotic culture for the reduction of Salmonella enteritidis in neonatal broiler chicks. Poult Sci 87: 27-31.

Mañes-Lázaro R, Van Diemen PM, Pin C, Mayer MJ, Stevens MP, Narbad A **(2017)** Administration of Lactobacillus johnsonii FI9785 to chickens affects colonisation by Campylobacter jejuni and the intestinal microbiota. Br Poult Sci 58: 373-381.

Methner U, Barrow PA, Martin G, Meyer H **(1997)** Comparative-study of the protective effect against *Salmonella* colonisation in newlyhatched SPF chickens using live, attenuated *Salmonella* vaccine strains, wild-type *Salmonella* strains or a competitive-exclusion product. Int J Food Microbiol 35: 223-230.

Nuotio L, Schneitz C, Halonen U, Nurmi E **(1992)** Use of competitive exclusion to protect newly-hatched chicks against intestinal colonisation and invasion by *Salmonella enteritidis* PT4. Br Poult Sci 33: 775-779.

Oh JK, Pajarillo EA, Chae JP, Kim IH, Yang DS, Kang DK **(2017)** Effects of Bacillus subtilis CSL2 on the composition and functional diversity of the faecal microbiota of broiler chickens challenged with Salmonella Gallinarum. J Anim Sci Biotechnol 8: 1.

Osimani A, Aquilanti L, Clementi F **(2016)** Salmonellosis associated with mass catering: a survey of European Union cases over a 15-year period. Epidemiol Infect 144: 3000-3012.

Palmu L, Camelin I **(1997)** The use of competitive exclusion in broilers to reduce the level of *Salmonella* contamination on the farm and at the processing plant. Poult Sci 76: 1501-1505.

Pascual M, Hugas M, Badiola JI, Monfort JM, Garriga M **(1999)** Lactobacillus salivarius CTC2197 prevents Salmonella enteritidis colonization in chickens. Appl Environ Microbiol 65: 4981-4986.

Saint-Cyr MJ, Haddad N, Taminiau B, Poezevara T, Quesne S, Amelot M, Daube G, Chemaly M, Dousset X, Guyard-Nicodème M **(2017)** Use of the potential probiotic strain Lactobacillus salivarius SMXD51 to control Campylobacter jejuni in broilers. Int J Food Microbiol 247: 9-17.

Schneitz C **(2005)** Competitive exclusion in poultry – 30 years of research. Food Control 16: 657–667.

Shibat El-hamd DM, Ahmed HM **(2016)** Effect of Probiotic on *Slamonella* enteritidis infection in broiler chickens. Egypt J Chem Environ Health 2: 298-314.

Smialek M, Burchardt S, Koncicki A **(2018)** The influence of probiotic supplementation in broiler chickens on population and carcass contamination with Campylobacter spp. - Field study. Res Vet Sci 118: 312-316.

Smith JM **(2014)** A review of avian probiotics. J Avian Med Surg 28: 87-94.

Stern NJ, Cox NA, Bailey JS, Berrang ME, Musgrove MT **(2001)** Comparison of mucosal competitive exclusion and competitive exclusion treatment to reduce *Salmonella* and *Campylobacter* spp. colonization in broiler chickens Poult Sci 80: 156-160.

Velhner M, Stojanov I, Potkonjak D, Kapetanow M, Orlić D, Rasic Z **(2005)** Salmonella enteritidis isolation from broiler chickens infected with low doses. Acta Vet-Beograd 55: 183-191.

Viscente JL, Torres-Rodriguez A, Higgins SE, Pixley C, Tellez G, Donoghue AM, Hargis BM **(2008)** Effect of a selected *Lactobacillus* spp.-based probiotic on *Salmonella enterica* serovar enteritidis-infected broiler chicks. Avian Dis 52: 143-146.

Wierup M, Wahlström H, Engström B **(1992)** Experience of a 10-year use of competitive exclusion treatment as part of the *Salmonella* control programme in Sweden. Int J Food Microbiol 15: 287-291.

Zebrowska JP, Witkowska D, Sobczak J, Mituniewicz T, Sowińska J **(2017)** Occurrence of Salmonella on poultry farms, in flocks of pigeons and feed monitored by the Department of Veterinary Hygiene in Olsztyn in the years 2014-2015. Med Weter 73: 111-117.