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THE EFFECTS OF PROBIOTICS ON *CAMPYLOBACTER JEJUNI* AND *SALMONELLA* SPP. WITH RESPECT TO THE MEAT AND THE ORGANS OF SLAUGHTERED CHICKENS

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Abstract: Our research deals with the effects of probiotics on *Campylobacter jejuni* and *Salmonella* spp. with respect to the meat and organs of slaughtered chickens. For the scope of our experiment, we used 250 one-day old chicks, divided into 5 groups. Initially, control chicken *group* was fed with feed not containing probiotics. Other groups were fed with feed containing different probiotics. Fattening-intended food was standardized for all groups. All chicken groups were exposed to the same ambient conditions. Following 42 days period of fattening, chickens were slaughtered. We took 30 samples of liver, intestine and swabs from perianal region for the needs of bacteriological examination. *Campylobacter* spp. and *Salmonella* spp. were determined by selective media. On the basis of obtained results, we can say that the application of probiotics in chicken feed reduces considerably the onset of *Campylobacter jejuni* and *Salmonella* spp. in meat and organs.

Key words: *Campylobacter* spp., *Salmonella* spp., broilers, probiotics

Introduction

The intestinal microflora is an integral part of the digestive system of all animals and chickens. Host-microbial interactions establish the eco-system of the animal. In the case of unstable intestinal microflora, biological defense from pathogens is considerably reduced.

Intestines of newborn animals are usually sterile *(Savage, 1987; Snoeybos et al.., 1978)*. In the case of adult animals, it does not take a long time for intestinal bacteria to develop from contaminated fecal material with which they come into contact. The transfer of bacteria from parents to the next generation is quite

efficient and enables newborn animals to develop protective intestinal flora in the first few days of their life *(Rolf, 1991)*.

Lineau et al. *(Lienau et al., 2003)* pointed out that *Campylobacter* spp. occurs in intestinal tract of healthy animals, but clinical symptoms of the disease need not necessarily be developed. At the farm level, microorganisms cause contamination in a variety of ways: via wild birds, as the consequence of insufficient cleansing and disinfection of cages intended to the transport of chickens and through contaminated on-farm water supplies.

Ivanovic *(Ivanović, 1991)* investigated the presence of *Campylobacter jejuni*/*coli* in caecum of slaughtered chicken. *Campylobacter jejuni*/*coli* were present in 94.3% of examined samples.

Salmonella spp. is also present in digestive system of chicken. *Rose et al. (1999)* examined the presence of *Salmonella* spp. at farms in France. The presence of bacteria was established in 70% of farms, even in one-day old chickens.
Japanese author *Murakami et al. (2001)* isolated *Salmonella* from the fecal material of chickens (34.7%) that originated from the farms and farm equipment (26.11%), eggs (18.6%) and of carcasses (37.8%).

Abouzeed et al. (2000) from Canada, isolated *Salmonella* from caecum of chickens in slaughtering line (32.5%).

The microorganisms in digestive system provide for a regular function of intestinal mucosa, facilitate digestion and stimulate motility of the system and immune response. Involved mechanisms are not completely explained, but commonly mentioned mechanism of action is "competitive exclusion" (CE).Competitive exclusion is the process in which non-pathogenic bacteria, naturally found in the mature animal's gastrointestinal (GI) tract are provided to newborn animals to prevent infection from pathogens *(Blankenship et al.,1990)*.

CE implies creation of environmental conditions that selectively favourise development of "good" bacteria (beneficial gut flora) and suppresses development of "bad" bacteria (pathogens), such as *Campylobacter* and *Salmonella* spp. The complexity of interaction makes rather difficult to research only one isolated factor. Many existing CE-included functional mechanisms are still unclear. The functions of the individual intestinal bacterial populations are unknown yet *(Sinovec et al., 1998)*. A way to trigger the onset of natural interaction of intestinal microbial flora is to apply probiotics. Microorganisms, used for animal feed, are usually gram positive bacteria of *Lactobacillus*, *Enterococcus*, *Pediococcus* and *Bacillus* genus. The mechanism of probiotic activity is not clear enough. It is considered that probiotics improve intestinal balance of digestive system: by toxins neutralization, by means of suppression of the growth of other microflora through the competition for adhesive receptors, eliciting metabolic disorder of other bacteria or by stimulation of an immune response. Probitoics are generally administered to animals through food or water. Daily feed intake is the best way to ensure

appropriately balanced probiotic quantities. Continuous feeding of animals with probiotics, over fattening period, provides for certain density of bacteria population in intestines. In the case of high intestinally-active bacterial concentration, a large amount of different substances is produced. Amino acids, vitamins and anti-microbial molecules are consequently produced in sufficient active amounts.

Guillot *(Gulliot, 2000)* supposes that the density of probiotic bacteria, lower than 10^7-10^9/g, does not suffice to establish a good balance between probiotics and intestinal bacteria. This opinion is important for respective host-related activity.

According to *Gaskin* (*1996*), friendly intestinal bacteria improve host immunity. Probiotics-originated bacteria may inhibit colonization of pathogens due to their ability that enables faster and stronger adhesion to epithelial cells, as in the case of *Lactobacillus acidophilus* in chickens that in that way eliminates *Salmonella infantis (Schneitz, 1993)*.

Materials and Methods

Description of chicken fattening process, probiotic composition and slaughtering procedure. For the scope of this Study, we used 250 one-day chickens of Arbor Acres species, of both sexes, of initial 40.07±0.33 g body mass. Chickens were divided into five groups. Each group consisted of 50 chickens. The fattening period was 42 days. Ambient conditions corresponded to prescribed technological standards for the subject type (BAA, ED 2030).

Holding conditions, feeding and watering were identical for all groups. Chickens were fed with complete feeding mixture of standard ingredients and standard chemical composition. Table 1 presents the composition of feeding mixture.

The composition of applied probiotic was as follows: **Probiotic 1** included**:** dry *Lactobacillus plantarum* fermented product, dry *Enterococcus* (*Streptococcus*) *faecium* fermented product, dry *Lactobacillus casei* fermented product and dry *Lactobacillus acidophilus* fermented product with minimal number of 1.0 x 10^7 CFU/g; **Probiotic 2** contained *Streptococcus faecium* - cernelle 68 in amount of 70 x 10^6 CFU/g; **Probiotic 3** contained 1 x 10^{10} CFU/g *Bacillus cereus* IP 5832 per 1 gram of product; **Probiotic 4** contained spores both of *Bacillus* CH 200, exceeding the quantity of 1.6 x 10^9 /g and spores of *Bacillus* CH 201 more than 1.6 x 10^9/g. The spores originated from two strains of bacillus: *Bacillus licheniformis* and *Bacillus subtilis* (1%).

Table 1. Feed composition for chicken

Component	**Composition of feed for chicken** (%)		
	Starter*	Grower*	Finisher
Corn	53.50	62.00	64.00
Sunflower meal	5.00	5.00	5.00
Soybean meal	26.00	18.50	18.00
Fish meal	4.00	2.00	-
Limestone	0.80	0.80	0.80
Dicalcyum phosphate	1.30	1.70	1.90
Fat	4.0	3.5	4.00
Salt	0.20	0.30	0.30
Premix	0.50	0.50	0.50
Yeast	2.00	1.00	1.00
Corn gluten	2.50	4.40	4.20
Methionin	0.20	0.15	0.15
Lysine	-	0.15	0.15
*Coccidiostatic (salynomycin-Na, Intervet) was added into feed (according to manufacturer)			

The control group No. 1 was fed with probiotic-free feed. The first experimental group (marked as No. 2) was fed with probiotic 1 enriched feed, added in the amount of 0.1 up to 0.05%. The second experimental group (marked as No. 3) was fed with Probiotic 2 enriched feed, added in the amount of 0.05%. The third experimental group (marked as No. 4) was fed with Probiotic 3-enriched feed, in the amount of 0.01 to 0.005% and the fourth experimental group (marked as No 5) was fed with Probiotic 4-enriched feed was in the amount of 0.05%.

Following the terminated fattening procedure, chickens were transferred to the pens of industrial slaughterhouse. Slaughtering and dressing were carried regularly. Carcasses were either air-chilled or water-chilled.

Sampling procedure. After evisceration, before washing of carcasses, 50 swabs were taken from perianal region, covering the surface 10 cm^2. Samples of liver and intestines were obtained from the same carcasses. Each sample was divided into two, placed into sterile plastic bag and kept in the refrigerator until the examination.

Isolation of Campylobacter spp. In our investigations we used liquid media prepared from Brucella broth in combination with antibiotics (*Ivanović, 2000*): polymyxin B 2500 IU, actidion 50 mg, rifampicin 5 mg and trimethoprim 5 mg. This medium is selective one since *Campylobacter spp*. are resistant to these antibiotics, but other bacteria species are not. In addition to liquid medium it is used solid medium by Skirrow (BioMerieaux).

Swab samples were inoculated in test tube containing liquid medium. Ten grams of previously macerated liver was placed into 10 mL of liquid medium preserved in sterile laboratory conditions. Scraped intestinal mucosa was inoculated in 10 mL of liquid medium.

Incubated liquid media were placed into container with microaerophilic atmosphere. Incubation lasted 48 h at + 43º C. Following the incubation period, the content of liquid medium was transferred to the medium by Skirrow, by means of inoculating loop, to be re-incubated under same conditions.

We used grown colonies for preparations, stained with 10% carbolfuxin and observed under microscope. The presence of *Campylobacter spp* was confirmed on the basis of its morphological properties. API Campy identification system was used for biotypization of isolated bacteria.

Isolation of Salmonella spp. For identification of *Salmonella* spp. we used also liquid and solid media. Selenite Cysteine Broth (Biolife) was used as a liquid medium and Xilose Lysine Desoxycholate Agar (XLD, Biolife), and Brilliant Green Agar (BG, Biolife) as solid media:

For the purpose of selective enrichment of *Salmonella* spp., swab samples were inoculated into 90 mL Selenite Cysteine Broth. Samples of macerated liver were inoculated into 90 mL of Selenite Cystine Broth. Samples from scraped mucosa were inoculated into 90 mL of Selenite Cystine Broth.

The broths were incubated at 37°C over the period of 24 h. Broth-obtained aliquots were spread, in duplicate, onto Xilose Lysine Desoxycholate Agar (XLD) and Brilliant Green agar (BGA) plates, and incubated at 37°C for 24 h. After plating, Selenite Cysteine Broths were returned to the incubator for another 24-hour period; in the process, they were plated out as described above. Suspect colonies on either XLD or BGA were confirmed using Gram staining, catalase, oxidase and urease tests, poly O anti-sera (Public Health Institute of Serbia).

Results and Discussion

Table 2 presents the results of *Salmonella* investigation in chickens.

In the first group, *Salmonella* spp. were detected in 42 swab samples taken from perianal region (84%), in 25 samples of liver (50%) and in 25 samples of intestinal mucosa (50%). The presence of *Salmonella* spp. in swab and liver

samples was not established in second group, but there were detected 5 cases (16%) in intestinal samples. The presence of Salmonella spp. in the third group was not determined. As for group 4, *Salmonella* spp. was identified in 17 swab samples taken from perianal region (34%), in 17 samples from liver (34%) and in 25 samples from intestinal mucosa (50%). Positive detection of *Salmonella* spp. in the fifth group was proved in the case of 17 swab samples (34%), in liver samples in 8 cases (16%) and in 17 samples from intestinal mucosa (34%).

Table 2. Probiotic influence on *Salmonella* spp. in chickens

Group Label	Samples								
	Swabs (perianal region)			Liver			Intestinal mucosa		
	No	Positive		No	Positive		No	Positive	
		No	%		No	%		No	%
1[a]	50	42	84	50	25	50	50	25	50
2[b]	50	0	0	50	0	0	50	8	16
3[c]	50	0	0	50	0	0	50	0	0
4[d]	50	17	34	50	17	34	50	25	50
5[e]	50	17	34	50	8	16	50	17	34

[a] – control group, feed without probiotic; [b] - feed with probiotic 1; [c] – feed with probiotic 2; [d] – feed with probiotic 3, [e] – feed with probiotic 4

Campylobacter spp. were presented in 34% liver samples in the first group. In the second group, *Campylobacter jejuni* was presented in 8 samples of liver (16%). The presence of *Campylobacter* spp. in the samples of the third, the forth and the fifth group was not determined, as indicated in Table 3. We detected *Campylobacter jejuni* in all positive samples.

Table 3. Probiotic influence on *Campylobacter jejuni* in chickens

Group Label	Samples								
	Swabs (perianal region)			Liver			Intestinal mucosa		
	No	Positive		No	Positive		No	Positive	
		No	%		No	%		No	%
1[a]	50	0	0	50	17	34	50	0	0
2[b]	50	0	0	50	8	16	50	0	0
3[c]	50	0	0	50	0	0	50	0	0
4[d]	50	0	0	50	0	0	50	0	0
5[e]	50	0	0	50	0	0	50	0	0

[a] – control group, feed without probiotic; [b] - feed with probiotic 1; [c] – feed with probiotic 2; [d] – feed with probiotic 3, [e] – feed with probiotic 4

In our work we examined the influence of probiotics upon different composition with respect to meat safety of chickens carcasses and the impact of different probiotics, in the presence of *Salmonella* spp. and *Campylobacter jejuni* that are rated as pathogens of significant Public Health concern.

Researchers of probiotic effects on microflora in poultry digestive system, indicate that probiotic-originated bacteria can inhibit the process of colonization with pathogenic bacteria, using their ability to bond faster and tighter to epithelial cells, as in the case of *Lactobacillus acidophilus*, thus eliminating *Salmonella infantis (Schneitz, 1993)*. Our results are in compliance with the referred literature statement. The Table 1 shows that the occurrence of *Salmonella* spp. was greater in the control chickens group fed with probiotic-free food. In some other groups, probiotic-fed chickens exhibited reduced *Salmonella* occurrence.

In the second group of chickens that were fed with probiotic 1. (*Lactobacillus plantarum*, *Streptococcus faecium*, *Lactobacillus casei* and *Lactobacillus acidophilus*), *Salmonella* appeared just in liver (16.16%). Those results comply well with the research of Impey et al. (*1982*), pointing out that pure cultures of Streptococci, *Lactobacillus*, Clostridia, *Bacteriodes* and *Bifidobacterium* can protect chicken from colonization with *Salmonella typhimurium* in some cases, in some other even provoked increase of these microorganisms in caecum.

Goren et al. (1984) pointed out that other bacteria, used in feed, can suppress pathogen growth. The application of *Bacillus subtilis* in chicken feed was rather challenging one, since it forms more stable spores at high temperatures in comparison to lactic acid type bacteria. Our results suggest that application of probiotic 3 (*Bacillus cereus* IP 5832) and probiotic 4 (*Bacillus* CH 200 and *Bacillus* CH 201) lower the percent of *Salmonella* presence in experimental groups, comparing to chickens from control group, but the percentage was higher than in chickens that got probiotic 1 (*Lactobacillus plantarum*, Streptococcus *faecium*, *Lactobacillus casei* and *Lactobacillus acidophilus*). Probiotic 2 (*Streptococcus faecium*) proved to be the most efficient for *Salmonella* reducing.

Baba et al. (1907) and *Newman* (*1996*) concluded that using *Lactobacillus* species, as nutritional additives, can diminish losses caused by salmonelosis, mortality and expansion of *Salmonella typhimurium* after infection. At the same time, other authors claim that probiotics application has not reduced *Salmonella typhimurim* on the samples taken from caecum and rectum *(Watkins et al., 1984)*.

Barnes (1979) tested the possibility of growth inhibition and multiplication of *Salmonella* species in vitro by application of 32 different types of anaerobic bacteria isolated from chicken intestines. *Bacterioides hypermegas* and *Bifidobacterium spp*. have consequently inhibited effect through the production of volatile fatty acids, creating unfavorable electrochemical reaction of intestinal tract for development of *Salmonella* species. By application of these bacteria in one-day chickens it is possible to prevent the infection with *Salmonella* typhimurium.

Nisbet et al. (1993) investigated the possibility of exclusion of *Salmonella typhimurim* by means of culture containing 11 food-added bacterial species, either with or without lactose and concluded that the protection against *Salmonella typhimurium* proved to be successful only in the case of lactose addition.

With respect to *Campylobacter jejuni* and on the basis of available literature resources, we have not managed to find out whether probiotics influence this microorganism in any specific way or not. We suppose that mechanism of influence is similar. As indicated in (Table 2), out of all presented samples, *Campylobacter jejuni* was recorded only in liver of the control group chickens (16.66%) (no probiotics in food) and in chickens that consumed probiotic 1. in their feed.

Conclusion

On the basis of our obtained results, the occurrence of *Campylobacter jejuni* and *Salmonella* spp. may be reduced significantly by application of probiotics-supplemented feed for chickens. *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus subtilis* produce the same effect of reduction on *Campylobacter jejuni*, comparing to *Streptococcus faecium* that produced lower reduction effect.

The best effect on reduction of *Salmonella* spp. was achieved by using probiotics containing monoculture of *Bacillus cereus* and monoculture of *Streptococcus faecium*. Lower effect was obtained with combined cultures of *Lactobacillus plantarum*, *Lactobacillus* casei, *Lactobacillus acidophilus*, *Streptococcus faecium* as well as *Bacillus licheniformis* and *Bacillus subtilis*.

Efekti probiotika na *Campylobacter jejuni* i *Salmonella* spp. u odnosu na meso i organe zaklanih brojlera

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Rezime

Naše istraživanje predstavlja efekte probiotika na *Campylobacter jejuni* i *Salmonella* spp. u odnosu na meso i organe zaklanih pilića. U eksperimentu je korišćeno 250 jednodnevnih brojlera, podeljenih u 5 grupa. Kontrolna grupa brojlera hranjena je smešama bez probiotika. Brojleri ostalih grupa hranjeni su smešama sa dodatkom različitih probiotika. Smeše za ishranu bile su standardne za svaku grupu, a brojleri svih grupa su držani pod istim uslovima. Posle 42 dana tova, brojleri su zaklani i uzeto je 30 uzoraka jetre, creva i briseva iz perianalne regije za bakteriološko ispitivanje prisustva *Campylobacter* spp. i *Salmonella* spp., Prisustvo ovih mikroorganizama je utvrđeno na selektivnim podlogama. Na osnovu dobijenih rezultata, može se reći da upotreba probiotika u ishrani brojlera redukuje *Campylobacter* spp. i *Salmonella* spp. u mesu i organima.

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