

EVALUATION LACTIC ACID BACTERIA AUTOSTRAINS WITH ANTI-CAMPYLOBACTER JEJUNI ACTIVITY ON BROILER CHICKENS PRODUCTIVITY

Yuri Kuznetsov^{1*}, Ilya Nikonov², Elena Kapitonova³,
Nadezhda Kuznetsova¹, Ruslan Omarov⁴

¹ Department of Parasitology, St. Petersburg State University of Veterinary Medicine, RUSSIA.

² Research Department, Perm State Agro-Technological University named after Academician D.N. Pryanishnikov, RUSSIA.

³ Department of Private Livestock, Vitebsk State Awarded the «Badge of Honour» Order Veterinary Medicine Academy, BELARUS.

⁴ Department of Technology of Production and Processing of Agricultural Products, Stavropol State Agrarian University, RUSSIA.

ARTICLE INFO

Article history:

Received 04 June 2020
Received in revised form 04
September 2020
Accepted 14 September 2020
Available online 21 September
2020

Keywords:

Cross Cobb-500; *C. jejuni*;
Campylobacteriosis;
Growing chickens;
Intestinal microbiota;
Zooanthroponotic
infections.

ABSTRACT

Modern poultry farming is based on preventing zooanthroponotic infections by targeted regulation of the intestinal microbiota using a wide range of probiotic preparations. This article reflects the results of a study on feeding broiler chickens with a feed additive containing autostrains of lactobacilli with anti-*Campylobacter jejuni* (*C. jejuni*) activity in comparison with intact chickens. As a result of the studies, autostrains of heterologous lactobacilli with activity against *Campylobacter* on the zootechnical parameters of growing broiler chickens "Cobb-500" was shown. Thus, the introduction of a feed additive based on lactobacilli, in general, had a positive effect on the productivity of broiler chickens.

Disciplinary: Agricultural and Animal Sciences, Microbiology; Bioscience.

©2020 INT TRANS J ENG MANAG SCI TECH.

1 INTRODUCTION

Modern poultry farming is based on preventing zooanthroponotic infections by targeted regulation of the intestinal microbiota using a wide range of probiotic preparations. The choice of the optimal probiotics for the prevention of campylobacteriosis in broiler chickens is relevant.

There are researches focused on the use of probiotics for the prevention and treatment of gastrointestinal tract infections caused by *Campylobacter jejuni* (*C. jejuni*) and *S. enteritidis*. In the works (Fooks and Gibson 2003; Fernández, et al., 2003), shown that symbiotic strains isolated from humans, *L. Plantarum* 0407 and *Bifidobacterium bifidum* Bb12, in the presence of oligofructose and

xylooligosaccharides inhibited the growth of *C. jejuni*, *in vitro* and *in vivo*. Similar results used lactobacilli and bifidobacteria isolated from chickens (Chaveerach et al., 2004). In Tsai et al. (2005), lactobacilli strains isolated from pigs and chickens exhibited antagonistic activity against *Salmonella spp* *in vitro* and *vivo*. Intact mobile flagella refer to the factors providing adhesion and survival of *C. jejuni* (Woodall et al., 2005). The flagellin locus contains two adjacent genes *fla A* and *fla B* (Hendrixson et al., 2001; Nuijten et al., 1990; Woodall et al., 20059). The intestinal environment, pH, viscosity, and various metabolites affect these genes (Sherman et al., 2009). Mucin stimulates the adhesion and internalization of *C. jejuni* into intestinal cells (Byrne et al., 2007; Szymanski, et al., 1995). The intestinal microaerophilic environment promotes the growth and reproduction of *C. jejuni*. The presence of probiotics inhibits the growth and reproduction of *C. jejuni* and prevents the penetration of the pathogen into the submucosal layer (Aguiar et al., 2013; Alemka et al., 2010). Exposure of *C. jejuni* cells to the probiotic impairs the pathogen's mobility and its ability to colonize the intestinal epithelium *in vitro* and *in vivo* experiments (Alemka, et al., 2010).

A comparative assessment of *B. subtilis* and *C. jejuni* under the conditions of the chicken gastrointestinal tract showed that *B. subtilis*, upon contact with *C. jejuni*, increases its mobility, reaches the crypts of the cecum more quickly, and occupies all binding sites. *B. subtilis* prevents the interaction of *C. jejuni* with the host organisms epithelial cells, limiting the mobility and survival of the pathogen (Aguiar et al., 2013). The addition of the probiotic feed additive Cellobacterin-T based on *B. subtilis* to broiler chickens' feed reduced the level of *C. jejuni* in the microbiota of the blind spines of the bird's gastrointestinal tract. A significant drawback of *B. subtilis* strains used as probiotics and probiotic feed additives is their feeble ability to adhere to the gastrointestinal tract's epithelial cells, in contrast to lactobacilli and bifidobacteria.

The study aimed to feed broiler chickens with a feed additive containing autostrains of lactobacilli with anti-*C. jejuni* activity in comparison with intact chickens.

2 MATERIALS AND METHODS

Broiler chickens of the Cobb 500 cross were rising within 35 days. The scheme of the experimental groups is in Table 1.

Table 1: Experiment scheme on broiler chickens

Group	Features of incubation and feeding of poultry
Control	Broilers chickens obtained after incubation of intact eggs. Feeding: basic diet
2	Broilers chickens obtained after incubation of eggs with introduced heterologous bacteria. Feeding: basic diet
3	Broilers chicks obtained after incubation of eggs with an injected sample without bacteria (violation of the egg). Feeding: basic diet
4	Broilers chickens obtained after incubation of intact eggs. Feeding: essential diet with the introduction of a feed additive containing heterologous lactic acid bacteria

For obtaining broiler chickens containing in the intestine strains of lactobacilli with activity against *Campylobacter*, into the incubation egg were introducing probiotic lactobacilli.

Chickens obtained after incubating intact eggs (without introducing bacteria) were used as a control group. Experiments on broiler chickens were in the vivarium of the International Laboratory of Molecular Genetics and Poultry Genomics (Federal State Budgetary Educational Institution of Higher Education "Moscow State Academy of Veterinary Medicine and Biotechnology - MBA named after K.I. Skryabin"). The ration of the breeder chickens was according to the recommendations of VNITIP (Fisinin et al, 2000; Egorov et al., 2019).

The chickens were in individual cages, observing the same technological parameters for growing (see Figure 1).



Figure 1: Chickens in individual cages, the same technological parameters of growing.

3 RESULTS AND DISCUSSION

Poultry feeding was in two phases (6-21 days and 22 days until the end of rearing). Complete feed was as the main diet for the experimental bird. Compound feed recipes are in Table 2.

Table 2: Recipes of compound feed for broiler chickens.

Ingredients	Age, days			Ingredients	Age, days		
	1-10	11-24	25-35		1-10	11-24	25-35
Yellow-grained corn	50.5	49.35	46.45	In 100 g of compound feed			
Wheat	6.00	-	-	Exchange energy, kJ	1268	1295	1307
Triticale	-	6.00	9.00	Crude protein,%	22.21	22.21	21.12
Soybean meal	30.00	31.00	27.00	Crude fiber,%	3.39	3.39	3.67
Sunflower meal	3.50	4.00	5.00	Crude fat,%	6.20	6.20	7.98
Fish flour	4.00	2.00	-	Calcium,%	1.08	1.08	1.04
Meat and bone meal	-	-	4.00	Phosphorus,%	0.76	0.76	0.78
Rapeseed oil	1.70	3.30	4.30	Sodium,%	0.17	0.17	0.18
Monocalcium Phosphate	1.30	1.20	1.25	Lysine,%	1.460	1.369	1.261
a piece of chalk	1.15	1.15	1.00	Methionine + cystine,%	1.072	1.030	0.988
Premix	2.00	2.00	2.00	Tryptophan,%	0.284	0.278	0.265

For the first five days, all groups' chickens received the same pre-starter compound feed by Ponomarenko et al., 2012. A feed additive containing lactobacilli for chickens of the 4th experimental group was starting from the 6th day of rearing. Chick weight results are in Table 3.

Table 3: Results of broiler chickens control weighing

Growing period	1st (control) group, g/head	2nd group, g/head	3rd group, g/head	4th group, g/head
1 (1-7 days)	212.6±4.32	258.3±3.24***	261.7±3.53***	269.3±3.54***
2 (8-14 days)	408.5±6.54	575.8±5.73***	593.5±5.32***	610.2±5.64***
3 (15-21 days)	751.4±8.63	970.8±7.31***	1005.3±7.75***	1030.6±7.64***
4 (22-28 days)	1264.8±10.87	1481.2±9.54 ***	1498.4±9.53 ***	1515.8±9.62 ***
5 (29-35 days)	1835.3±13.96	2091.0±11.46 ***	2100.1±11.54 ***	2150.5±11.31 ***

Note: * $P > 0.95$; ** $P > 0.99$; *** $P > 0.099$.

The safety of broiler chickens in all groups was 100%. Indicators in Table 2 reflect that in all periods of growing broiler chickens of the 4th group, significantly exceeded the analogs of the control group in live weight and the analogs of the 2nd and 3rd experimental groups.

The results of the consumption of feed by control and experimental groups are in Table 4.

Table 4: Feed consumption per 1 kg of live weight gain of broiler chickens

Indicators	Group			
	1	2	3	4
Feed consumption per 1 kg of growth for the entire growing period, kg	1.98	1.88	1.86	1.81
By % to control	100	94.9	93.9	91.4
Feed conversion, kg	-	0.10	0.12	0.17
By % to control	-	5.1	6.1	8.6

Feed consumption per 1 kg of live weight gain of "Cobb-500" cross broiler chickens in all experimental groups decreased in comparison with the 1st control group, in the 2nd group by 5.1%, in the 3rd group by 6.1%, and in the 4th group by 8.6%.

4 CONCLUSION

The introduction of lactobacilli into eggs generally had a positive effect on reared broilers' performance. The introduction of under-shell strains of lactobacilli with anti-*C. jejuni* activity and incubation of eggs for 21 days to obtain broiler chickens containing lactobacilli autostrains in the intestinal caecum with anti-*C. jejuni* activity is promising for the prevention of campylobacteriosis.

5 AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding authors

6 ACKNOWLEDGEMENT

The study was performed at the FGBOU VO "St. Petersburg State Academy of Veterinary Medicine» with the aid of the Russian Science Foundation Grant, Project No. 18-76-10017.

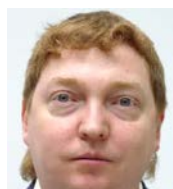
7 REFERENCES

- Aguilar, V.F., Donoghue, A.M., Arsi, K., Reyes-Herrera, I., Metcalf, J.H., de los Santos, F.S., Blore, P.J., Donoghue, D.J. (2013). Targeting motility properties of bacteria in the development of probiotic cultures against *Campylobacter jejuni* in broiler chickens. *Foodborne Pathog Dis.*, 10(5), 435- 441.
- Alemka, A., Clyne, M., Shanahan, F., Tompkins, T., Corcionivoschi, N., Bourke, B. (2010). Probiotic colonization of the adherent mucus layer of HT29MTXE12 cells attenuates *Campylobacter jejuni* virulence properties. *Infect Immun.*, 78(6): 2812-2822.
- Byrne, C.M., Clyne, M., Bourke, B. (2007). *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells in vitro. *Microbiology*, 153, 561-569.
- Chaveerach, P., Lipman, L.J.A., van Knapen, F. (2004). Antagonistic activities of several bacteria on in vitro growth of 10 strains of *Campylobacter jejuni/coli*. *Int J Food Microbiol.*, 90(1), 43-50.

- Egorov, I.A., Manukyan, V.A., Okolelova, T.M., et al. (2019). *Poultry feeding guide*. Sergiev Posad: VNITIP. 226.
- Fernández, M.F., Boris, S., Barbés, C. (2003). Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J Appl Microbiol.*, 94(3), 449-455.
- Fisinin, V.I. (2000). *Feeding poultry*. Sergiev Posad. 375.
- Fooks, L.J., Gibson, G.R. (2003). Mixed culture fermentation studies on the effects of synbiotics on the human intestinal pathogens *Campylobacter jejuni* and *Escherichia coli*. *Anaerobe*, 9(5), 231-242.
- Hendrixson, D.R., Akerley, B.J., DiRita, V.J. (2001). Transposon mutagenesis of *Campylobacter jejuni* identifies a bipartite energy taxis system required for motility. *Mol Microbiol.*, 40, 214-224.
- Nuijten, P.J., van Asten, F.J., Gaastra, W., van der Zeijst, B.A. (1990). Structural and functional analysis of two *Campylobacter jejuni* flagellin genes. *J Biol Chem.*, 265, 17798-17804.
- Ponomarenko, Yu.A., Fisinin, V.I., Egorov, I.A. (2012). Safety of Feed, Feed Additives, and Food. Minsk. *Ecological perspective*, 864.
- Sherman, P.M., Ossa, J.C., Johnson-Henry, K. (2009). Unraveling mechanisms of action of probiotics. *Nutr Clin Pract.*, 24(1), 10-14.
- Szymanski, C.M., King, M., Haardt, M., Armstrong, G.D. (1995). *Campylobacter jejuni* motility and invasion of Caco-2 cells. *Infect Immun.*, 63(11), 4295-4300.
- Tsai, C.-C., Hsieh, H.-Y., Chiu, H.-H., Lai, Y.-Y., Liu, J.-H., Yu, B., Tsen, H.-Y. (2005). Antagonistic activity against *Salmonella* infection in vitro and in vivo for two *Lactobacillus* strains from swine and poultry. *Int. J. Food Microbiol.*, 102, 185-194.
- Woodall, C.A., Jones, M.A., Barrow, P.A., Hinds, J., Marsden, G.L., Kelly, D.J., Dorrell, N., Wren, B.W., Maskell, D.J. (2005). *Campylobacter jejuni* gene expression in the chick cecum: evidence for adaptation to a low oxygen environment. *Infect Immun.*, 73, 5278-5285.



Yuri Kuznetsov is an Associate Professor. He is a Candidate of Veterinary Sciences. His research includes Veterinary Immunology and Stress Pharmacology, Increasing the Effectiveness of Drugs, and studying the Side Effects of Drugs and Reducing their Negative Impact on the Body.



Ilya Nikonov is Deputy Director for Science and Development, BIOTROF LLC. Senior Researcher of the Scientific and Technical Information Department of the All-Russian Scientific Research Veterinary Institute of Poultry (VNIVIP), St. Petersburg. His research deals with the study of the intestinal microflora of poultry, Feed, Habitat. He is a developer of the composition and methods for assessing the Effect of Antimicrobial Feed Additives (Probiotics, Prebiotics, Phytobiotics, etc.) and Mycotoxin Sorbents.



Elena Kapitonova is an Associate Professor at the Department of Private Livestock, Vitebsk Order of the Badge of Honor State Academy of Veterinary Medicine. She is a Candidate of Agricultural Sciences Her research interests are the Development of Feed Additives, the Technology of Raising Animals (Birds), Feeding Farm Birds.



Nadezhda Kuznetsova is an Associate Professor. She is a Candidate of Veterinary Sciences. Her scientific interests are Pharmacological Corrections of Animal Productivity, an Alternative to Feed Antibiotics.



Ruslan Omarov is an Assistant of Professor at the Department of Technology of Production and Processing of agricultural products, Stavropol State Agrarian University, Russia. He is a Candidate of Technical Sciences. His researches are Meat, Meat Products, Farm Animal Blood.