

**Comparative studies of the effect of applied probiotics  
LAKTIFERM BASIC 300<sup>®</sup> and LAKTINA<sup>®</sup> on survival and mortality  
in pheasants infected with *E. COLI* O 103**

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**Abstract**

*The effect of the application of probiotics Laktiferm Basic<sup>®</sup> 300 and Laktina<sup>®</sup>, and antibiotics Colivet Oral powder<sup>®</sup> and Pharmastim 8 %<sup>®</sup> at pheasants were compared. We explored the possibility of use of probiotics for the prevention and treatment of E. coli infections in pheasants aged from 1 to 30 days. In the experiment take part 5 groups pheasants infected with enteropathogenic strain of E.coli O 103 in an amount of 10<sup>8</sup> cfu / ml ( positive control - treated with Colivet Oral powder<sup>®</sup>, negative control and three test groups with: Laktiferm Basic 300<sup>®</sup>, Laktina<sup>®</sup> and Pharmastim<sup>®</sup>). Survival, mortality and post-mortem lesions in infected with E. coli pheasants of all groups were studied.*

**Key words:** probiotics, pheasants, E.coli, post-mortem lesions, Laktiferm Basic 300<sup>®</sup>, Laktina<sup>®</sup>

**INTRODUCTION**

In 2006 The European Union imposed a complete ban on antibiotic growth promoters in all types of animal foods. Pre-and probiotics appeared as an alternative and promising solution to the nutritive banned antibiotics. The use of probiotics is objectively determined by a number of positive effects on the body. Since the beginning of the century in many countries conduct in-depth studies to selection and use of specific types of beneficial probiotic organisms to regulate the ecological balance in the intestinal tract, which would result in a stable microbial population with a strong antagonistic activity against pathogenic microorganisms.

Under the conditions of intensive livestock production is difficult to maintain a balance in the gastrointestinal tract of an animal, since they are exposed to many stress factors: congestion in animal houses, sudden changes in environmental conditions and diet, and the treatment with other antibiotics. These factors led to the dominance of harmful microflora and cause reduced feed conversion, a drop in the

growth of fattened animals, diarrhea, increased susceptibility to secondary infections and death. Some authors have made experimental studies on the inhibitory effects of lactic acid bacteria, and show that in the absence of these microorganisms in the gut is disrupted, degradation of the proteins in the results in the formation of non-resorbable component, which contribute to the growth of harmful micro-flora and occurrence of enteritis (Annuk al., 2003; Dunne al., 1999).

These findings raise the idea of lactic acid bacteria and / or their metabolites can be administered orally in the form of feed additives. From this arises the concept of probiotics as food supplements for prophylaxis of gastrointestinal disorders in animals and foster growth and growth through better feed conversion (Penkov al., 2004; Penkov al., 2004). Positive effect on growth, feed utilization and health of broiler chickens using probiotics produced by lactic acid bacteria and yeasts, some authors reported (Chotinsky al. 2002). Other authors on the basis of numerous experiments with birds treated with probiotics reported to improve productive performance,

maintaining a normal and beneficial microflora in the digestive tract through antagonism and competitive exclusion, neutralization of enterotoxin and stimulate local immunity in the intestine and others (Alexieva al., 2004; Georgieva al., 2006; Ignatova al. 2004; Vahyen al, 2002).

While poultry is some research with probiotics with strong positive effect it at hunting birds object farmers breed and raise such studies are extremely scarce.

We aimed to investigate survival, mortality, pathology and histopathology changes in pheasants infected with a pathogenic strain of E.coli, compared the effects of probiotics Laktiferm Basic<sup>®</sup> 300 and Laktina<sup>®</sup> with antibiotics Colivet Oral powder<sup>®</sup> and Pharmastim 8%<sup>®</sup>.

## MATERIALS AND METHODS

Were purchased 40 pheasants at 1 day old from state game breeding station - Chekeritsa. All the pheasants were included in one experiment with the separation of the chicks in 5 groups of 8 numbers in the group, to participate in the experiment. On the 3rd day all groups of pheasants were inoculated per oral (intra ingluvial) with enteropathogenic strain of E.coli O 103 in an amount of 10<sup>8</sup> cfu / ml.

The pheasants of the five groups were treated as follows: positive control group - receive a supplement to water (colistin sulfate) Colivet<sup>®</sup> 1 g per 1 liter water; negative control group - no additives; I experimental group - added Laktiferm Basic 300<sup>®</sup> 0.5 g probiotic per 1 kg feed; II experimental group - added Laktina<sup>®</sup> 0.5 g probiotic per 1 liter of water; III experimental group - added Pharmastim 8%<sup>®</sup> 2 g nutritional antibiotic to 1 kilogram feed.

Was prepared compound feed for chickens without the addition of commercial nutritional antibiotic (substitution made by us probiotic or nutritional antibiotic).

Experiment is conducted under the conditions in the vivarium of the Faculty of Veterinary Medicine in the corpus "D" in Studentski Grad, Sofia starting on July 1, 2012. During the 30-day experiment was conducted following observations:

Survival and mortality - dead pheasant during the experiment were recorded promptly and after autopsy samples were taken from the bodies for post-mortem observations. (Intestine, liver, kidney and spleen)

### Description of antibiotics and probiotics:

Antibiotic **Colivet Oral powder<sup>®</sup>** (Seva Animal Health Bulgaria) contains: Colistin (sulphate) 1.2 MIU / g.

Antibiotic **Pharmastim 8%<sup>®</sup>** (BIOVET, Peshtera, Bulgaria) contains: FLAVOPHOSPHOLIPOL (bambermitsin) - 8,0 g.

Probiotic **Laktiferm Basic 300<sup>®</sup>** (Chr. Hansen, Czech Republic) contain: Enterococcus Faecium M74 in 1g of not less than 300 x 10<sup>9</sup>CFU / g.

Probiotic **Laktina<sup>®</sup>** (Lactina Ltd., Bankya, Bulgaria) contains: Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus casei, Bifidobacterium longum, Lactobacillus acidofilus tpc in 1g of not less than 1 billion.

\*-dosage of antibiotik **Colivet<sup>®</sup>** in dose 1,0g/ 1 drinking water recommend buy the CEVA Animal Health. - Bulgaria

\*\*-dosage of probiotic **Laktiferm Basic 300<sup>®</sup>** in dose - 0,5 g/kg (0,5 kg/t) recommend buy the Chr. Hansen, Czech Republic

\*\*\*-dosage of probiotic **Laktina<sup>®</sup>** in dose - 0,5 g /l drinking water recommend buy the Laktina Ltd. - Bulgaria

\*\*\*\*- dosage of antibiotik **Pharmastim 8%<sup>®</sup>** in dose - 2 g / kg recommend buy the (BIOVET, Peshtera) Bulgaria

Table 1. Design of experiment:

Groups Parameters	positive control group (Colivet <sup>®</sup> )	negative control group	I experimental group (Laktiferm Basic 300 <sup>®</sup> )	II experimental group (Laktina <sup>®</sup> )	III experimental group (Pharmastim 8% <sup>®</sup> )
Starter feed (1-30 day)	combined forages for pheasants + antibiotik Colivet <sup>®</sup> in dose 1,0g/ 1 drinking water *	combined forages for pheasants	combined forages for pheasants + probiotik Laktiferm Basic 300 <sup>®</sup> in dose 0,5 g/kg forage **	combined forages for pheasants + probiotik Laktina <sup>®</sup> - 0,5 g /l drinking water ***	combined forages for pheasants + antibiotik Pharmastim 8% <sup>®</sup> in dose - 2 g / kg forage ****

## RESULTS AND DISCUSSIONS

As a result of study of survival and mortality found that died during the experiment pheasant were divided into groups as follows: positive control group - receiving an additive to the water of (colistin sulfate) Colivet® at 1 g per 1 liter of water, survival - 50% and mortality - 50%; negative control group - no additives, survival - 12.5% and mortality - 87,5%; I experimental group - added Laktiferm Basic 300® at 0.5 g probiotic per 1 kg feed, survival - 50% and mortality - 50%; II experimental group - added Laktina® at 0.5 g probiotic per 1 liter of water, survival - 37.5% and mortality - 62.5% ; III experimental group - added Pharmastim 8%® at 2 g nutritional antibiotic per 1 kg feed, survival - 25% and mortality rate - 75% , as presented and figure (figure 1).

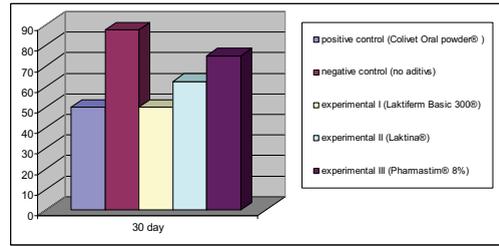


Figure 1. Mortality (%) during the growing period

In the course of our study, we found various pathological and pathohistological changes. Pathologists following changes: the abdomen is bloated, the entire abdominal wall is affected by a moist gangrene (maceration) (Figure 2), local and diffuse peritonitis (Figure 3a and 3b), highly swelling (ballooning) small intestine filled with liquid and gas, and hyperemia of the liver (Figure 4), enlarged spleen with petechial haemorrhages and diffuse peritonitis (Figure 5), the caeca are pale and distended, that are overfilled with fluid containing many gas bubbles (Figure 6).



Figure 2. The abdomen is bloated, the entire abdominal wall is affected by a moist gangrene (maceration).

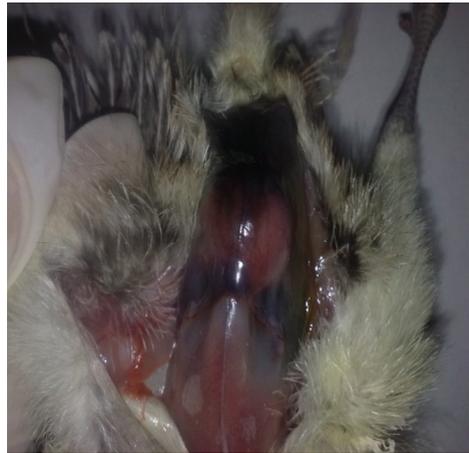


Figure. 3a Diffuse peritonitis.

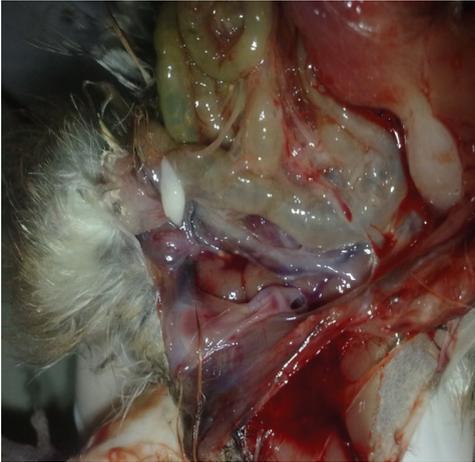


Figure.3b Local peritonitis.



Figure 4. Highly swelling (ballooning) small intestine filled with liquid and gas, and hyperemia of the liver.

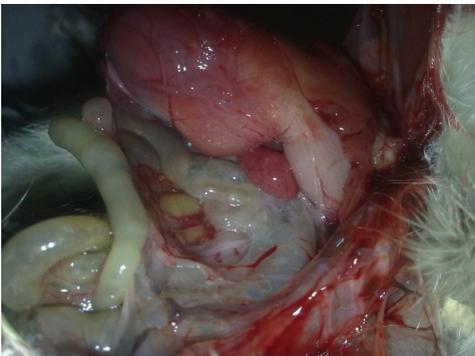


Figure 5. Enlarged spleen with petechial haemorrhages and diffuse peritonitis.



Figure 6. The caeca are pale and distended, that are overfilled with fluid containing many gas bubbles.

In conducting histopathological examination found: many erythrocytes in propria of villi intestinal (Figure 7 a,b); expanded and filled with erythrocytes hepatic sinus capillare (Figure 8 b); Under capsular hemorrhage in spleen (Figure 9); hemorrhage in the kidney interstitium (Figure 10).

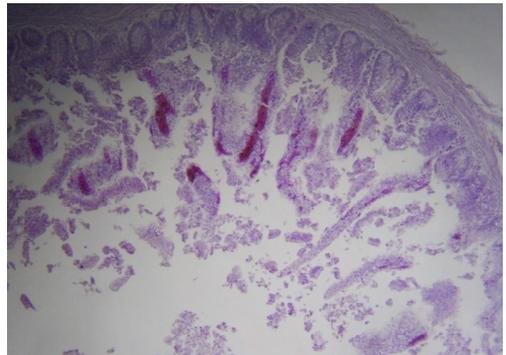


Figure 7a. Many erythrocytes in propria of villi intestinal x25

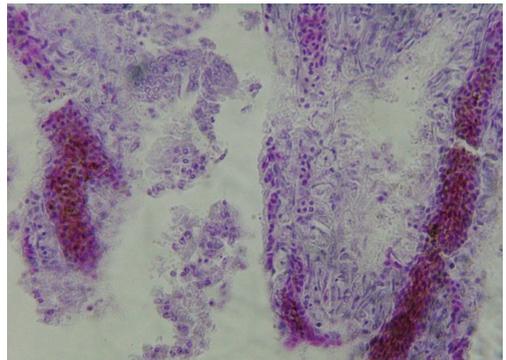


Figure 7b. Many erythrocytes in propria of villi intestinal x40

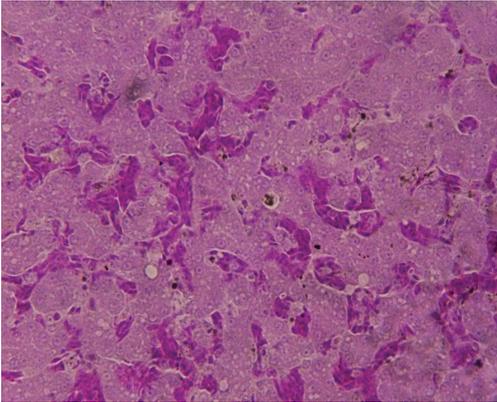


Figure 8. Expanded and filled with erythrocytes hepatic sinus capillare x 40

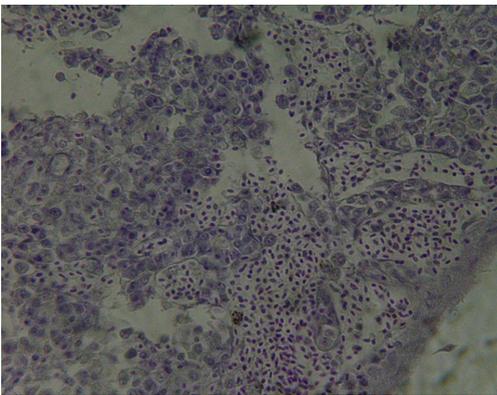


Figure 9. Under capsular hemorrhage in spleen x 40

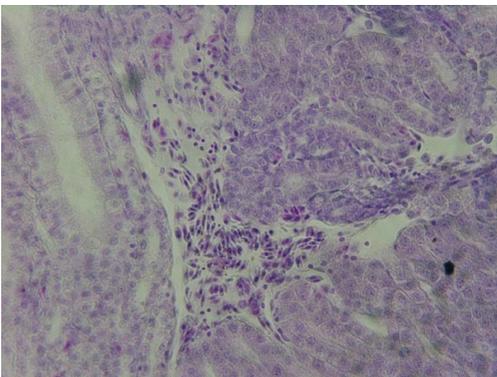


Figure 10. Hemorrhage in the kidney interstitium x 40

Analyzing our results of the study came to the conclusion that there is a tendency to reduce the mortality rate of birds from the experimental groups treated with antibiotics and Laktiferm Basic 300<sup>®</sup>, compared to the negative control group. These results tend to be

due to the fact that enterococci (*Enterococcus Faecium*) metabolism of conduct etc. "erratic" type ferment various hydrocarbons to form primarily lactic acid, but not gas, lowering the pH to 4.2 to 4.6, unlike the lactobacilli (*Lactobacillus bulgaricus*) which maintain a pH of 5.5 to 5.6 level (<http://evkoma.com>). Considering that coliforms (*Escherichia coli*) live in an environment with an optimal pH 6.7 to 8, and do not grow at pH 4-5, probiotic Laktiferm Basic<sup>®</sup> 300 has a better effect of probiotic Laktina<sup>®</sup> with *E. coli* infections in pheasants (Andrew, 2008). Similar results obtained and other authors, which compare the effect of probiotic CLOSTAT<sup>®</sup> with antibiotic Colistin<sup>®</sup> in broiler chickens infected with a pathogenic strain of *Escherichia coli* (Teo al, 2006).

## CONCLUSIONS

Although not statistically significant credibility, there is a tendency to reduce the mortality rate of birds from the experimental groups treated with antibiotics and Laktiferm Basic 300<sup>®</sup>, compared to the negative control group.

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