

STUDY OF PERFORMANCE ON NATURAL RESISTANCE IN PHEASANTS, INFECTED WITH PATHOGENIC STRAIN OF *E.COLI* AND TREATED WITH *ENTEROCOCCUS FAECIUM* M74

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Abstract

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Probiotics are live micro-organisms that bring benefits to consumer health. It is well known that they are used for prevention of bacterial diseases in poultry, as well as to potent the growth performance and immune response of poultry.

The present work was carried out to evaluate the effectiveness of probiotic Laktiform Basic 300® on natural resistance in pheasants, infected with pathogenic strain of *E.coli*.

Pheasants were inoculated intra ingluvial with enteropathogenic strain of *E.coli* O 103 in an amount of 108 cfu/ml on the 3rd day and divided into four equal groups: Group (1) served as a positive control group, received colistin sulfate. Group (2) was negative control. Group (3) was fed with the probiotic while group (4) was fed with nutritional antibiotic Pharmastim 8%®. Evaluations were made for immunological changes. Our results showed that Enterococcus Faecium M74 possesses positive effect on phagocytic activity and on bactericidal activity of blood serum. Significant decrease was recorded in the phagocytic percent and phagocytic index of neutrophils of pheasants in negative control.

Keywords: pheasant, probiotic, Enterococcus Faecium M74, phagocytic activity, bactericidal activity of blood serum

Introduction

Probiotics, the novel feed or food supplements, are naturally occurring live microorganisms that bring benefits to consumer health. Immunomodulatory effects of intestinal microflora is generally recognized (Gaggia et al., 2010), although the mechanism of action of probiotics on the mucosa is not fully understood (Loddi, 2003; Ng et al., 2009).

Innate immunity is the first line of antimicrobial host defense in most multi-cellular organisms, and is instructive to adaptive immunity in higher organisms. Probiotics act on the non-specific immune response and on the adaptive immune response (Toms and Powvie, 2001; Koenen et al., 2004).

The problem of the natural resistance of chicks to disease and adverse environmental factors are the subject of investigations of a number of authors. Non-specific defense mechanisms and adaptation ability as a rule proved almost immediately after hatching. Phagocytic activity, the action of com-

plement and lysozyme, bactericidal activity of the serum, etc. are one of the most important protection criteria.

Study the performance of innate immunity are the basis for analysis and the health of the birds and to respect the rules of welfare (Padgett and Glaser, 2003; Broom, 2006).

- Heterophils isolated from broiler chickens, treated with *B. subtilis* PB6, showed increased phagocytic activity against *E. coli* (Teo and Tan, 2007). Sadeyen et al. (2004) demonstrated that inbred strains birds, susceptible to *Salmonella*, induce lower expression of IFN- γ in the cecal form tonsils, compared to resistant.
- The importance of present study lies in the fact that probiotic bacteria are used as immunomodulators but a literature survey revealed that the effect of probiotics have not been studied for immune response of pheasants. The purpose of current study was to investigate the changes of natural resistance in pheasants, infected with pathogenic strain of *E.coli* and treated with Enterococcus Faecium M74.

Materials and Methods

Experimental Design

Thirty-two one-day old pheasants were obtained from the the state game breeding station - Chekeritsa.

On the 3rd day all pheasants were inoculated per oral (intra ingluvial) with enteropathogenic strain of *E.coli* O 103 in an amount of 108 cfu/ml. **Pheasants were divided into 4 groups** of 8 birds each:

- positive control group – receive a supplement to water (colistin sulfate) Colivet® 1 g per 1 L water;
- negative control group - no additives;
- first experimental group - added Laktiferm Basic 300® 0.5 g probiotic per 1 kg feed;
- second experimental group - added Pharmastim 8%® 2 g nutritional antibiotic to 1 kg feed.

The feed for chickens was compound without the addition of commercial nutritional antibiotic. The pheasants were housed in a floor pen. Feed and water were provided ad libitum.

On the 30^d day for immunological analyses blood samples were taken in a syringe with added heparin from the heart of the pheasants.

Probiotic

Laktiferm Basic 300® (Chr. Hansen, Czech Republic) contains: Enterococcus Faecium M74 in 1g of not less than 300 x 10⁹CFU/g.

Cellular factors

Cellular factors of innate immunity were studied by determining the phagocytic activity and phagocytic index of polymorphonuclear leukocytes.

The percentage of phagocytosis indicates percentage of neutrophils which have phagocytosed latex particles by the total number (200) of neutrophils listed in smears.

- Percentage of phagocytosis = No. of ingesting phagocytes/ Total number of phagocytes including non ingesting cells.

Phagocytic index reflects the average number of latex particles phagocytosed by one neutrophil.

- Phagocytic index = No. of ingested *C. albicans* cells/No. of Ingesting phagocytes.

Humoral factors

Humoral factors of innate immunity are investigated determining bactericidal activity of blood serum. Bactericidal activity was determined by the method of Saharuhanov et al. (2007) In the study 50 µl plasma and the period of incubation of 90 minutes were used. The suspension of the test - system accounted for 300 µl (1 billion microbial bodies in 1

ml sample) and the volume of broth - 2 ml. The bactericidal activity was determined by the formula:

$$\bullet \text{ \% of the bactericidal activity} = 100 - \frac{O_{90} - O_0}{K_{90} - R_0} \times 100$$

where O_{90} - optical density of the test sample after 90 minutes; O_0 - an optical density of the test sample before incubation; K_{90} - an optical density of the control sample after 90 minutes; R_0 - an optical density of the control sample before incubation

Statistics

The collected data were statistically analysed by using STATMOST.

Results and Discussion

The phagocytic ability of neutrophils is determined – see Figure 1.

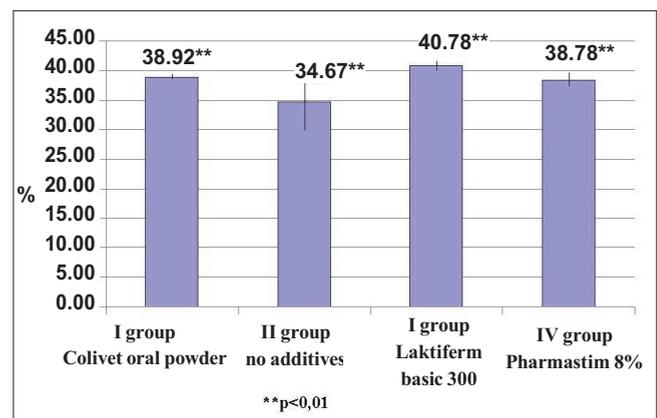


Fig. 1. Phagocytic activity of polymorphonuclear leukocytes of pheasant, infected with enteropathogenic strain of *E. coli* treated with probiotics and antibiotics against gastrointestinal diseases. Results are presented as mean ± SE

The reliability of the results obtained in all experimental groups was $p < 0.01$. The percentage of neutrophils expressing phagocytic activity in birds from the control group was 34.7 ± 4.8 , and it increased in the pheasants, received Lactiferm - up to $40.7 \pm 0.78\%$. Higher in comparison with the control is the percentage of phagocytic activity in the groups treated with an antibiotic and in those who received as an additive Pharmastim 8%, respectively 38.9 ± 0.5 and 38.4 ± 1.2 .

The same trend was observed in the results determining the phagocytic index (Figure 2).

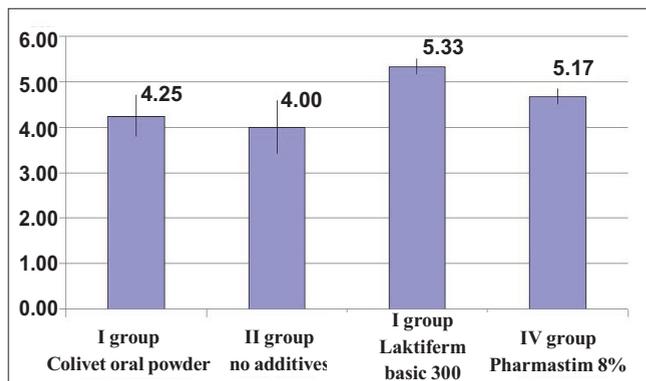


Fig. 2. Phagocytic index of polymorphonuclear leukocytes from the peripheral blood of pheasants. Results are presented as mean ± SE

The highest phagocytic index of neutrophils in whole blood pheasant was detected in birds in a third group (5.3 ± 0.2). In comparison between groups statistically significant differences were not observed. The chart shows that this indicator has a dynamic change in the positive and negative controls.

Phagocytosis is one of the oldest and most important mechanisms of non-specific protection. It is known that as the process of phagocytosis and the functional activity of phagocytes, are sensitive to the action of factors of the external and internal environment.

Infection caused by *E.coli*, do not induce changes in the immune reactivity in one-week-old chicks, when bacterial antigen invades the body 9 days before vaccination (Nakamura et al., 1986). There is only lymphocytic depletion of lymphoid tissue. Our data of decreased phagocytic activity and lower phagocytic index in the negative control correlate with data (Hegazy et al., 2010), showing that in younger birds immune system is suppressed and establishes lower postvaccination titers and phagocytic index. Krukowski and Smith (2005) confirm that chronic bacterial and fungal infections suppress neutrophil function.

The bactericidal activity of blood serum is an integration factor of the humoral mechanisms of natural resistance, which characterizes the ability of the organism to self-cleaning.

The survey results of this indicator in our study are shown in Figure 3.

The trials found the lowest rate of bactericidity in pheasants from the first group $23 \pm 2.0\%$. Values in the other groups ranged from $26 \pm 1.2\%$ in the second to 33.6 ± 4.3 - in the third test group, as the statistical accuracy of the results has not been established.

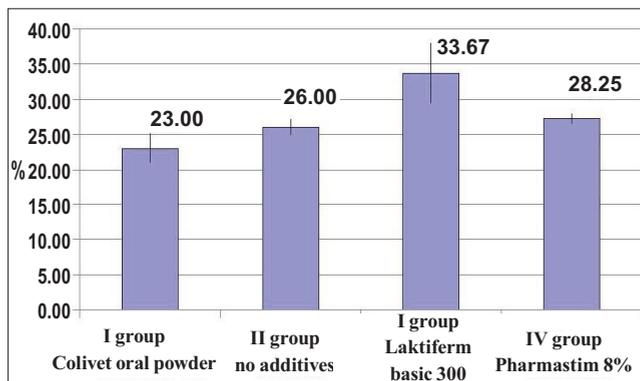


Fig. 3. Bactericidal activity of blood serum. Results are presented as mean ± SE

The bactericidal power of the blood is directly related not only with the complement system and the natural antibodies but also to the presence of β -lysine, and lectin (Kawasaki et al., 1989). It is proved that this parameter of the non-specific resistance increases with the age of the birds. (Franciosini et al., 2011). Our research was on the 30-day pheasants, which gives us no reason to make such conclusions at this avian species.

Conclusion

The study of the non-specific defense shows that those mechanisms are essential in the initial period of development not only for poultry, but and for pheasants, when adaptive mechanisms do not show its essence.

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