

## COMPARATIVE STUDY ON THE EFFECT OF LACTINA® PROBIOTIC ON SOME MICROBIOLOGICAL AND HISTOLOGICAL CHARACTERISTICS OF THE DIGESTIVE TRACT OF MUSCOVY DUCKLINGS

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### ABSTRACT

A study on the influence of Lactina probiotics on the content of some microbial species and some histological characteristics in the intestine of Muscovy ducklings has been conducted.

The authors proved, that Lactina probiotic had a significant effect on the microflora in the large intestines, suppressing the development of the enterococci and pathogenic bacteria of Salmonella species and stimulating the development of the lactic acid bacteria.

The structure of the intestinal wall corresponded to an active functional status, no significant differences being established in the histological structure between the experimental and the control groups.

**KEYWORDS:** Muscovy ducks, probiotics, microbiological, histological indexes

### РЕЗЮМЕ

Проведено е изследване на влиянието на пробиотик Лактина върху съдържанието на някои микробиални видове и някои микробиологични характеристики на микробиалния тракт на Мускусни патета. Авторите доказват, че пробиотикът Лактина влияе значително върху микрофлората дебелите черва, като подтиска развитието на ентерококи и патогенни бактерии от род Salmonella и стимулира развитието на млечнокисели бактерии. Структурата на тънките черва има активен функционален статус, като не са открити значителни разлики в хистологичния строеж между контролната и опитната групи.

**КЛЮЧОВИ ДУМИ:** Мускусни патици, пробиотик, микробиологични, хистологични показатели.

## DETAILED ABSTRACT

Lactina<sup>®</sup> probiotic produced in Bulgaria contains lyophilizing strains of *Streptococcus* and *Lactobacillus* species. The standardized product contains CFU - min  $1.10^8$ /g and lactic acid 2,0 - 2,6 %. Its stimulating effect was studied in fattening pigs and exerted a high stimulating effect on the growing parameters of young breeder ducklings, as well as on the amino acid content of their breast muscle.

The aim of the present study was to establish the changes in the ratio between the major groups of microorganisms and the histological structure of the intestine tract of growing Muscovy ducklings fed with and without Lactina<sup>®</sup> probiotic additive.

In 2003 an experiment was conducted with 108 Muscovy ducklings (white variety) distributed into 3 groups, 36 birds in each group, by the method of analogues – sex and live weight. The first group was fed without probiotics, the second group was fed diet, containing probiotics Lactina – 1 kg/t diet, from 1-28 day of age, 0.5 kg/t diet from 29-70 day of age, and the 3 group 0.5 kg/t diet only from 29-70 day of age.

Samples were taken from them for the content in their large intestines and samples for the histostructure of their small and large intestines.

When reporting the content of the separate groups of microorganisms in the large intestines, an obviously expressed tendency of decreasing the number of bacteria of *Enterobacter* genus was observed in the fowl receiving Lactina probiotic (from the first as well as from the 28<sup>th</sup> day) in comparison with the control. As for the microorganisms of *Lactobacillus* genus, they were high in number for the fowl receiving the probiotic from the first and from the 28<sup>th</sup> day and comparatively low in number for the fowl from the control group – Table 1, Figure 1. It was established that pathogens of *Salmonella* genus were not found in the intestine content of ducklings given the probiotic, while in the group given it from the 28<sup>th</sup> day and especially in the control group, single numbers of that genus were to be noticed.

The histological study showed that Lamina epithialis mucosae in the small intestines of the Muscovy ducklings from the control and the experimental groups was represented by a single-layer prismatic absorption epithelium and single cup-shaped cells excreting mucus. The intestinal glands had a diameter of 65-70  $\mu$ m and the height of the structural gland epithelium cells was about 20 $\mu$ m. The intestinal histological structure of both experimental groups did not differ from the one established for the control.

Lactina probiotic had a significant effect on the microflora

in the large intestines, suppressing the development of the enterococci and pathogenic bacteria of *Salmonella* genus and stimulating the development of the lactic acid bacteria.

The structure of the intestinal wall corresponded to an active functional status, no significant differences being established in the histological structure between the experimental and the control groups.

## INTRODUCTION

Probiotics were discussed as an alternative to the nutritive antibiotics in feeding agricultural animals and poultry [3]. They are biostimulators containing live or lyophilizing bacterial cultures, which regulate and optimize the ratios among the different types of microorganisms in the digestive system, preventing upsets and exerting a stimulating effect on the disintegration and absorption of the nutrient substances, as well as on some microbiological and histological characteristics of the digestive tract [4].

Lactina<sup>®</sup> probiotic produced in Bulgaria contains lyophilizing strains of *Streptococcus* and *Lactobacillus* species. The standardized product contains CFU - min  $1.10^8$ /g and lactic acid 2,0 - 2,6 %. Its stimulating effect was studied in fattening pigs [11] and broilers [8]. The probiotic exerted a high stimulating effect on the growing indices of young ducklings bred for reproduction [5], as well as on the amino acid content in their breast muscle [6].

The aim of the present study was to establish the changes in the ratio between the major groups of microorganisms and the histological structure of the intestine tract of growing Muscovy ducklings fed with and without Lactina<sup>®</sup> probiotic additive.

## MATERIAL AND METHODS

In 2003 an experiment was conducted with 108 Muscovy ducklings (white variety) distributed into 3 groups, 36 birds in each group, by the method of analogues – sex and live weight.

The ducklings in the different groups were bred under the same conditions without any additional influence on the microclimate: until the 14<sup>th</sup> day – indoors with an area of 3 m<sup>2</sup> for each group, from the 15<sup>th</sup> until the 28<sup>th</sup> day – an area of 4.5 m<sup>2</sup>. From the 29<sup>th</sup> to the 70<sup>th</sup> day the ducklings were bred in voliers with an area of 6 m<sup>2</sup> indoors and yards in the open for free access in daytime with an area of 10 m<sup>2</sup> and water area of 4 m<sup>2</sup>.

Feeding scheme: Experimental diets were made in the poultry farm. All the components were submitted to chemical and amino acidic analyses. The content of

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metabolizable energy was calculated using the mean coefficients of digestibility of the nutrient substances [1] and the apparent metabolizable energy equation of Schiemann et al. [7]. Two-phase ad libitum feeding was carried out according to the following scheme:

Starter diet: (0 – 28<sup>th</sup> day) – maize – 70 %, sunflower meal – 15 %, soybean meal - 11 %, mineral-and-vitamin premix for poultry (Biovet – Peshtera) - 1.5 %, salt - 0.5 %, chalk - 1 %, dicalcium phosphate - 1 %. 1 kg diet contained: AME - 11.9 MJ, crude protein – 16.57 %, lysine – 0.7 %, methionine + cystine - 0.45 %, Ca- 0.82 %, P (total)- 0.6 %, crude fibres - 5.1 %.

Finisher diet: (29<sup>th</sup> – 70<sup>th</sup> day) - maize – 75 %, sunflower meal – 15 %, soybean - 6 %, mineral-and-vitamin premix for poultry (Biovet – Peshtera) - 1.5 %, salt - 0.5 %, chalk - 1 %, dicalcium phosphate - 1 %. 1 kg diet contained: AME - 12.1 MJ, crude protein – 14.99 %, lysine – 0.6 %, methionine + cystine - 0.45 %, Ca- 0.82 %, P (total)- 0.54 %, crude fibres – 4.6 %.

During the whole period of study the ducklings from the first group (control) were fed diets without Lactina® probiotic. For the ducklings of the second group the probiotic was added at a rate of 1 kg/t of diet until the 28<sup>th</sup> day and at a rate of 0.5 kg/t of diet after the 28<sup>th</sup> day, while for those from the third group – only after the 28<sup>th</sup> day at a rate of 0.5 kg/t of diet.

After the end of the experiment 3 male and 3 female ducklings were selected from each group with the live weight close to the mean weight of the group.

Samples were taken from them for the content in their large intestines and samples for the histostructure of their small and large intestines.

The microbiological analyses were carried out by the indirect method of Coch [cited by 12], using the selective nutrient media for: Salmonella bact. Sp. – agar of Endo and brilliantgrune-phenolrot-agar; Enterobacter sp. (E. coli)– agar of Endo, blood agar and tripaflavine-agar; Lactobacillus sp.– whey and milk agar. From 1 g of the intestine content we prepared dilutions of 1:10 to 1:10 000 with sterile physiological solution. 0.1 cm<sup>3</sup> was taken from each dilution and it was spread on the respective nutrient medium /in two repetitions at least/, and then cultivated at a temperature of 30-37<sup>o</sup> C for 24 – 48 hours. The specific belonging of the colonies was confirmed by microscope studies after staining by Gram.

The materials for the histological study were taken from the last third part of the small intestine for all the three groups of ducklings. The parts were fixed in 10 % solution of neutral formalin and then we followed the procedure by [9] – inclusion in paraffin, cutting by microtom to slices of 6 micrometer thickness, staining

with hematoxillin-eosin. The preparations were observed and pictures were taken by a universal microscope NU, magnifying 10x40.

### RESULTS AND DISCUSSION

When reporting the content of the separate groups of microorganisms in the large intestines, an obviously expressed tendency of decreasing the number of bacteria of Enterobacter genus was observed in the fowl receiving Lactina probiotic (from the first as well as from the 28<sup>th</sup> day) in comparison with the control. As for the microorganisms of Lactobacillus genus, they were high in number for the fowl receiving the probiotic from the first and from the 28<sup>th</sup> day and comparatively low in number for the fowl from the control group – Table 1, Figure 1. It was established that pathogens of Salmonella genus were not found in the intestine content of ducklings given the probiotic, while in the group given it from the 28<sup>th</sup> day and especially in the control group, single numbers of that genus were to be noticed.

A similar tendency was also reported by [10] in experiments with chickens given pure yeast of Lactobacillus acidophilus and by [2] who summarized that the probiotic preparations containing more and varied lactic acid microorganisms manifested higher inhibiting effect to enteropathogenic microorganisms in comparison with the pure strains.

It can be concluded that the active bacterial substances of Lactina probiotic suppress the development of the enterobacteria as well as of the conditional pathogenic and pathogenic bacteria such as those of Salmonella genus.

The histological study showed that Lamina epitalis mucosae in the small intestines of the Muscovy ducklings from the control and the experimental groups was represented by a single-layer prismatic absorption epithelium and single cup-shaped cells excreting mucus. The intestinal glands had a diameter of 65-70 µm and the height of the structural gland epithelium cells was about 20µm. The intestinal histological structure of both experimental groups did not differ from the one established for the control – Figure 2.

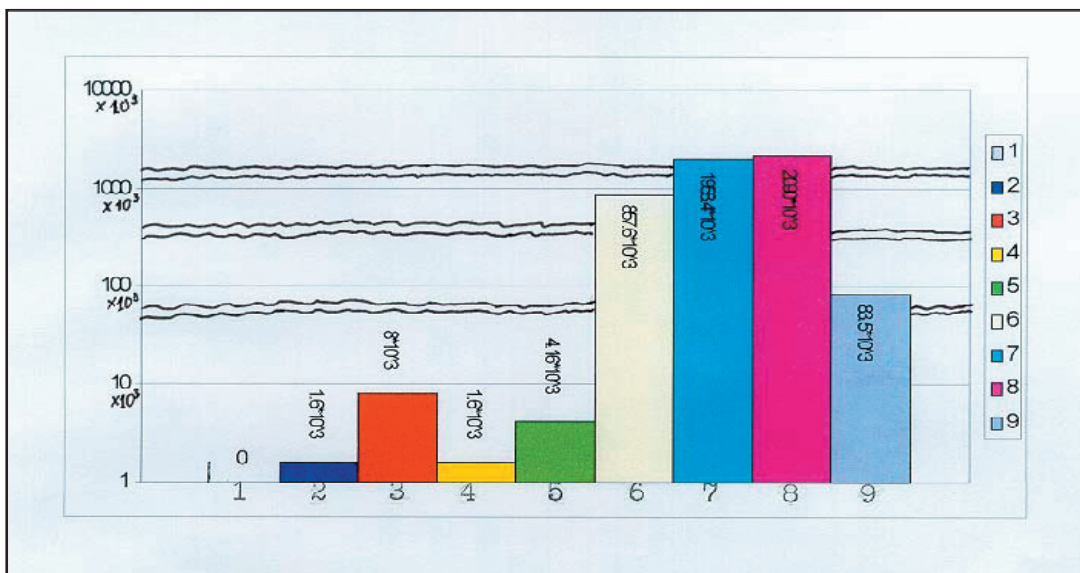
### CONCLUSIONS

Lactina probiotic had a significant effect on the microflora in the large intestines, suppressing the development of the enterococci and pathogenic bacteria of Salmonella genus and stimulating the development of the lactic acid bacteria.

The structure of the intestinal wall corresponded to an

Table 1. Total number of bacteria in the hindgut of Muscovy ducklings (number/ml)

Groups→ Bacterial cultures ↓	With probiotic from 1 <sup>st</sup> day	With probiotic from 28 <sup>th</sup> day	Without probiotic
Salmonella- sp.	-	1.6*10 <sup>3</sup>	8*10 <sup>3</sup>
Enterobacter- sp.	1.6*10 <sup>3</sup>	4.16*10 <sup>3</sup>	857.6*10 <sup>3</sup>
Lactobacilus- sp.	1958.4*10 <sup>3</sup>	2080*10 <sup>3</sup>	83.5*10 <sup>3</sup>



Legend:  
 1-Salmonella- sp. -Probiotics from 1-st day of age; 2- Salmonella- sp. - Probiotics from 29nth day of age; 3-Salmonella- sp. -control group  
 4- Enterfbacter- sp. - Probiotics from 1-st day of age; 5-Enterfbacter- sp. - Probiotics from 29nth day of age; 6- Enterfbacter- sp. -control group  
 7- Lactobacilus- sp.- Probiotics from 1-st day of age; 8- Lactobacilus-sp.- Probiotics from 29nth day of age; 9- Lactobacilus- sp.- control group

Figure 1. Comparative data about the availability of different species.

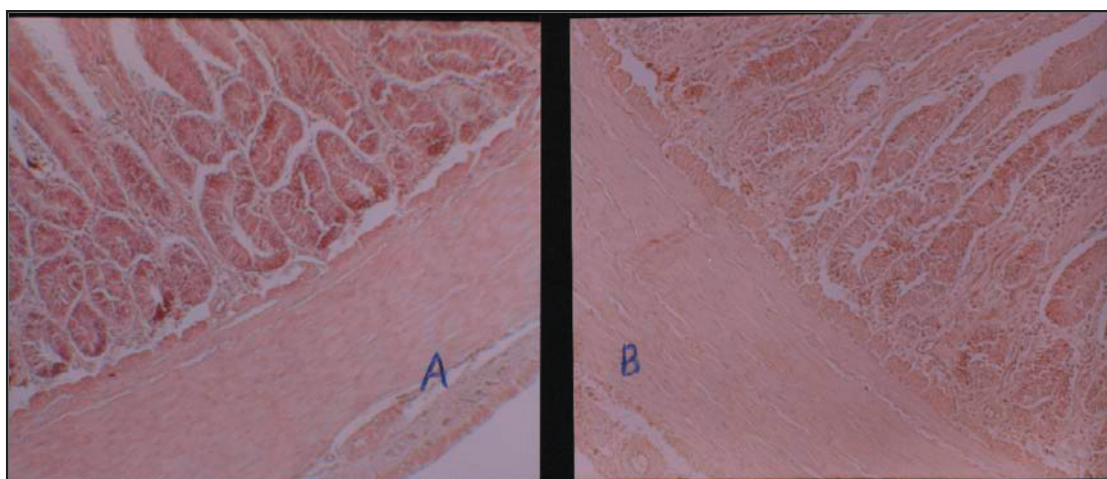


Figure 2. Cross-section of small intestines of 70-day old ducklings (A – control group, B – group given Lactina probiotic from the first day).



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active functional status, no significant differences being established in the histological structure between the experimental and the control groups.

### ACKNOWLEDGEMENT

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