

CHANGES IN THE GUT MICROBIOTA OF BROILER CHICKENS AFTER *LACTOBACILLUS* SP. PROBIOTIC TREATMENTS: A MINI-REVIEW

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Abstract

Biotechnology studies have documented several positive effects of probiotic products on the animals' state of health. Probiotic *Lactobacillus* sp. treatments applied to broiler chickens may help restore the balance of the gastrointestinal microbiota. The way probiotics act against pathogens can prevent microbial diseases and improve the host's resistance to gut infections during physiological stress occurring in the intensive production system. Other advantages for the host expected from the use of probiotic products as feed additives are the synthesis of vitamins and the activation of the immune system. According to morphological approaches, there are some changes in the size of the intestinal absorption surface recorded after the administration of probiotic treatments to the broiler chickens. Such histological changes would relate to a better assimilation of the feed. Increasing the nutrient availability by the lactic acid bacteria colonies in the gastrointestinal tract improves the broiler chickens' production. The probiotic treatments are associated with a reduction in the number of pathogen populations and an increased percentage of the beneficial microorganisms, both leading to a better health status of the host. The applicability of probiotic properties *in vivo* depends on keeping the appropriate ratio between the concentration of the probiotic products and the feed quantity adapted to the intense growth needs of the broiler chickens.

Key words: probiotics, *Lactobacillus* sp., microbiota, broiler chickens, gastrointestinal tract

INTRODUCTION

As part of the animal science research programs, the use of probiotics in poultry production is a potential alternative to antibiotics' [11], which covers the safety concerns for the chicken meat consumers. The effects of these additives on broiler chickens' state of health are well documented by experiments leading to the growth promotion of beneficial bacteria and the reduction in the number of pathogens in the gastrointestinal tract (GIT) [15].

The addition of diluted samples of pure *Lactobacillus* sp. cultures into the broiler chickens' diet may serve as a means of covering the appropriate nutrients demand through symbiotic processes in the gut. It is also associated with morphological changes

at the intestinal mucosa level due to the development of lactic acid bacteria (LAB). There have been carried out several histological investigations after probiotic experimental treatments applied to pathogen-challenged broiler chickens, which provide evidence for the usefulness of probiotics for the gut regeneration [5], [18].

MATERIALS AND METHODS

Preliminary in vitro analyses

The potential probiotics sources made up of LAB are cultivated *in vitro* in order to determine some parameters by laboratory tests. Such biotechnological investigations actually simulate the occurrence of the specific effects during the entrance of *Lactobacillus* sp. strains into the GIT of the host and the subsequent localization in its organism [2]. Data regarding the concentration changes in the tested microbiological samples are usually obtained

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by the spread plate technique, which uses a solid medium [MRS (de Man, Rogosa and Sharpe) agar] [14], [16]. This technique is more appropriate for the live microbial cells counting compared to the spectrophotometric method. The number of microbial cells [CFU (colony forming units)] / mL is expressed by the product between the mean number of colonies (a) and the corresponding dilution level with changed sign (10^n) divided to the volume of inoculant (V):

$$\text{CFU} / \text{mL} = a \times 10^n / V$$

Determination of the species of probiotic microorganisms

Confirming the taxonomic characters at the species level in strains with previously characterized phenotype involves several analyses of the fermentation profile. Such analyses consist of the determination of enzymatic potential in relation to the carbohydrate substrates available in the API (Analytical profile index) kits Bio-Merieux [17].

The taxonomic classification of microbiological samples is based on molecular DNA extractions which are investigated by PCR (polymerase chain reaction) method and, further, by electrophoresis. The DNA samples present specific taxonomic differences which can be detected in the molecular structure. This methodology is applied in order to identify the isolated strains at the species level [14], [16].

Administration of the probiotic treatments in broiler chickens

For the inclusion of probiotic products in the drinking water of the broiler chickens, for treatment purposes, the samples must be previously diluted at the ratio of 10^9 microbial cells / mL, which gives the appropriate concentration [18].

RESULTS AND DISCUSSION

Stress occurring in the poultry production system is responsible for the reduction of beneficial bacteria populations (i.e. *Lactobacillus* and *Bifidobacterium* species) which inhabit the broiler chickens' gut. The

probiotic therapy seems to have a positive influence both on birds' immunity [9] and on gut microflora [10]. Keeping a balanced microbiota in broiler chickens raised in an intensive system is a potential way to control certain pathogens localised in the gut, such as *Escherichia coli* [8] or *Salmonella* [12] and to increase the host's resistance to infectious diseases. The inclusion of probiotics in the broiler chickens' diet may stimulate the development of the LAB colonies in the GIT and improve the state of health [18].

The physiological condition of the chickens following probiotic treatments may be positively changed by an increased potential of beneficial bacteria including *Lactobacillus* sp., *Bifidobacterium* sp., and *Enterococcus* sp. and a reduction of the survival rate in pathogens i.e. *Campylobacter jejuni* [7] and *E. coli* [4], [13]. The influence of probiotic treatments on the growth of LAB populations in ileum and caeca may be recorded around the ages of 21 and 42 days in broiler chickens corresponding to optimal growth periods [3].

Other benefits of probiotics treatments for the birds' organism consist of increasing digestive efficiency, improving the natural defence system against pathogens by means of symbiotic relationship and vitamin synthesis [1].

There are several experimental studies which describe the effects of some probiotic strains of *Lactobacillus* sp. on the gut microflora, either alone or in combination with different LAB. The corresponding articles contain data concerning the increasing counts of beneficial bacteria and the drop in the number of pathogens after probiotic treatments applied to broilers. Other research experiments refer to *Bacillus* sp. as a reference strain for probiotic products, which has similar effects on the gut microbiota composition as in the case of *Lactobacillus* sp.: decreasing the harmful bacteria and enhancing the beneficial microbes [13].

The results of the above-mentioned papers are listed below:

- A probiotic treatment associated with yoghurt did not affect the broiler chicken's ileal and caecal bacterial

counts at day 42, but reduced the caecal aerobes and *E. coli* at day 21 [9].

- A probiotic product *L. acidophilus* included in the diet of broiler chickens improved gut health of the experimental groups. The bacterial counts in ileum and caeca at days 21 and 42 suggested a stimulation of the growth in LAB populations and an inhibitory effect on Coliforms and total anaerobes in the groups fed probiotics [3]
- A probiotic strain, *Lactobacillus rhamnosus* CF tested on broiler chickens improved intestinal microflora in chickens more efficiently than *Enterococcus faecium*. The caecal content of *Escherichia coli* decreased while that of *Lactobacillus* sp. increased in values [4].
- The dietary supplementations with *Lactobacillus acidophilus* D2/CSL in rurally reared chicken breeds significantly increased the population of *Lactobacillus* sp. but had no significant influence on the populations' size of *Enterococci*, *Staphylococci*, and *Escherichia coli*. Those probiotic treatments seem to have exerted a near-significant inhibitory effect on coliforms, instead [6].
- In a multi-strain probiotic experimental treatment applied to chickens challenged with food-borne pathogens there were recorded significant reductions in the caecal colonies of *Campylobacter jejuni* compared to control treatments [7].
- There were carried out several experimental studies on the effects of probiotic sources different from *Lactobacillus* sp. (i.e. *Bacillus* sp.) which proved to be satisfactory for their activity of reducing the pathogenic bacteria in the gut. After a dietary treatment consisting of a combination of organic acids and probiotics there was noticed an improvement of the gut microflora in broiler chickens. The microbiological contents of the gut expressed a trend of increasing the *Lactobacillus* sp. and decreasing the *E. coli* counts [13].

CONCLUSIONS

The beneficial changes occurring inside the broiler chickens' organism by the addition of probiotic products to the diet are based on preserving gastrointestinal microbiota balance. According to several experimental studies, broiler chickens improve their health state and become more productive under appropriate conditions of probiotics administration.

Due to the symbiosis ecology, the LAB which make up the probiotic products bring higher nutritional properties to the feed and help the optimal functioning of the digestive system by antimicrobial activity. The broiler chickens' organism benefits from an increased percentage of symbiotic LAB capable of making certain nutrients more available and suffers morphological changes responsible for the increase in the intestinal absorption surface.

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