Slovak J. Anim. Sci., 39, 2006 (3): 155 - 159

COLONIZATION OF GASTROINTESTINAL TRACT OF TURKEYS AFTER PROBIOTICS AND PREBIOTICS APPLICATION

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ABSTRACT

This project investigates the microbiological and biochemical characteristics in ceca of turkey. Microbiological characteristics were represented by CFU of *Escherichia coli, Enterococcus sp.* and *Lactobacillus sp.* determined in 1 g of chyme and biological ones by enzymatic activity of the cellulases expressed in CMC units. In all experiments, probiotic preparation based on *Enterococcus faecium* and prebiotic preparation based mannanoligosacharides were applied into feeding mixture and drinking water at various concentrations. Counts of *Escherichia coli* CFU in 1 g of cecal chymuse were determined on McConkey agar, counts of CFU of *Enterococcus sp*. on Slanetz-Bartley agar and counts of *Lactobacillus sp.* on MRS agar. Enzymatic activity of cellulases was determined according to the Miller method. Counts of CFU of *Escherichia coli, Enterococcus sp., Lactobacillus sp.* and enzymatic activity of cellulases were compared in experimental and control treatments, respectively. Theory and empirical evidence suggest that the counts of *Escherichia coli* CFU would be higher in control treatments and CFU counts of both faecal enterococci and lactobacilli as well as enzymatic cellulases activity (in CMC units) lower in control treatments compared to the experimental ones. Similar results were also achieved in our experiments with turkey. Polymerase Chain Reaction (PCR) method using specific primers was used to identify *Enterococcus faecium* bacterium.

Key words: caecal chyme; enzymatic activity of cellulases; *Escherichia coli*; *Enterococcus sp.*; *Lactobacillus sp.*; probiotic and prebiotic preparation; turkeys

INTRODUCTION

The intestinal microflora is an integral part of the digestive system of animals. The fact that they are living organisms means that they have nutritional and spatial requirements. Bacteria in the gastrointestinal tract derive most of their energy for reproduction and growth from dietary compounds which are either resistant to attack by digestive fluids or absorbed so slowly by the host that bacteria can successfully compete for them. Since bacterial species differ from each other in relation to their substrate preferences and growth requirements, the chemical composition and structure of the *digesta kartely* determines the species distribution of the microbial community in the gastrointestinal tract. As a consequence, bacterial community structure is very much dependent upon the diet as the ultimate source of substrates for metabolism (Savory, 1992; Wagner and Thomas, 1987). The bacterial community at a given point of time, therefore, reflects the capability of each bacterial group to compete against other bacterial groups and the defense system of the host in the prevailing chemical and physical environment. Vice versa, the ability of the host digestive system to digest and absorb nutrients is, in part, dependent upon the species distribution and total population of resident microbes. Hence, changes in dietary composition or nutrient density can have dramatic effects on the intestinal microfloral populations (Gibson et al., 1996; Hillman, 1999; Reid and Hillman, 1999), which in turn can influence the ability of the animal to digest and absorb dietary nutrients.

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Probiotic bacteria have been defined as "live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance" (Fuller, 1989). Probiotic bacteria are increasingly utilized in human food as well as in animal feed products (Fuller, 1999; Sanders and Huis in't Veld, 1999). However, composition of the intestinal microbiota is poorly known, which hinders the understanding of the probiotic functions (Tannock, 2005). A probiotic strain should be of host origin, non-pathogenic, technologically suitable for industrial processes, acid- and bile-fast, adhere to the gut epithelial tissue, persist in the gastrointestinal tract for short periods, produce antimicrobial substances, modulate immune responses and influence the metabolic activities of the gut (Dunne et al., 1999). The properties of the strain should be well documented (Dunne et al., 1999). Although some criteria, such as the non-pathogenic status, technological suitability and careful documentation of the probiotic effects of a microbial strain, are invariably required, no single strain is likely to carry all of the above mentioned properties (Dunne et al., 1999). Moreover, probiotic properties are considered strain-specific, and results obtained with one strain cannot therefore be claimed for another, even a closely related strain. Microbes used in probiotic products include strains from several *Lactobacillus* and *Bifidobacterium species, Enterococcus faecalis* and *Enterococcus faecium, Lactococcus lactis, Leuconostoc mesenteroides, Pediococcus acidilactici,* and *Sporolactobacillus inulinus, Streptococcus thermophilus*, as well as *Bacillus cereus, Escherichia coli, Propionibacterium freudenreichii, Saccharomyces cerevisiae* and *Saccharomyces boulardii* (Holzapfel et al., 1998).

Our experiments with turkey focused on testing probiotic and prebiotic preparations in which the active probiotic substance was represented by Enterococcus faecium, the milk fermentation bacteria and active prebiotic substance was represented by mannanoligosacharides.

We investigated the antagonism of *Enterococcus faecium* against *Escherichia coli* bacteria; its positive effect on propagation of lactobacilli and stimulation of enzymatic activity which would have a positive influence on metabolism of the host macroorganism.

MATERIAL AND METHODS

Quantitative microbiological and biochemical analysis.

Applied methods:

PLATE DILUTING METHOD

Determination of CFU counts

Plate diluting method was applied for quantitative CFU counts (CFU - colony forming units) for determination of respective groups of microorganisms in 1 g of substrate.

Gelatinous nutritive substrate in Petri dishes was inoculated with 1 ml of chyme samples by flushing (*Lactobacillus sp., Escherichia coli, Enterococcus sp.*) in three replications. Homogenized samples of caecal chyme (chyme was used to sterile Petri dishes) were prepared in advance by sequential dilution based on decimal dilution system application.

Dilution of the samples

Basic dilution (10^{-2}) was prepared as follows: 1 g of caecum content was added to the bank containing 99 ml of distilled water. The cells were separated from substrate by scutching in shaking machine (30 minutes). The basic substance prepared in this way was diluted to reduce the content of microorganisms at the level of less than 300 CFUs.

Applied nutritive substrates:

Three sorts of nutritive substrates for cultivation and specification of respective groups of microorganisms were applied (table 1). The compositions of these nutritive substrates were in accordance with the directions for use as prescribed by the producer (IMUNA, n.p. Šarišské Michaľany).

Investigation of enzymatic activity of the cellulases was carried out according to the Miller method (1960). Cellulase activity (from 1% carboxymethylcellulose) was measured by the method of releasing reducing sugars. Results were expressed in CMC units.

Table 1: Isolated species of microorganisms and their fundamental identification signs (Holt et al., 1994)

Cultivated species	Nutritive substrate	Temperature of cultivation	Time of cultivation	Colour of the colony
Escherichia coli	McConkey agar	37°C	24 to 48 hours	pink
Enterococcus sp.	Slanetz-Bartley agar	37°C	48 to 72 hours	red
Lactobacillus sp.	MRS agar	37°C	72 hours	light amber

VERIFYING TRIALS

In the trial with turkeys, the quantitative representation of microorganism groups in caeca of turkeys was studied after the application of probiotic preparation based on *Enterococcus faecium* M-74 and prebiotic preparation based mannanooligosaccharides. The trial was realized on experimental basis at the Department of Poultry Keeping and Small Farm Animals at the Slovak Agricultural University in Nitra. Samples were collected from 12 weeks old turkeys. Fattening itself went on from 1 to 84 days of the age of turkeys. One-day old turkeys of Large White hybrid were randomly distributed to 5 groups as follows:

1. treatment - control without probiotic and prebiotic application,
2. treatment - addition of prebiotic preparation at the quantity of 2 $kg.t^{-1}$(0,2 %) into feed,
3. treatment-addition of prebiotic preparation at the quantity of 1 $kg.t^{-1}$ (0,1 %) into feed
4. treatment-addition of probiotic preparation at the concentration of 5.10^9 $cfu.g^{-1}$ into feed daily
5. treatment-addition of probiotic preparation at the concentration of 5.10^{10} $cfu.g^{-1}$ into water in graded amount by the age and weight from 2.10^8 to 30.10^8KTJ.g^{-1}.

During the trials, turkeys were stabled in one-storey cages. Microclimatic conditions were maintained at the level of large-scale production parameters. During the course of the trial, the following feeding mixtures were used:

- HYD 12 (for first two weeks);
- HYD 13 (from the beginning of the 3rd to the end of the 7th week);
- HYD 14 (from the beginning of the 8th to the end of the 11th week).

POLYMERASE CHAIN REACTION (PCR)

The polymerase chain reaction was used to confirm the unambiguous presence of *Enterococcus faecium* (applied into digestive tract) in the group of *Enterococci*.

DNA isolation from caecal chymuse of chicken

About 0.5 g of chime sample was homogenised in 1.5-3 ml of physiological solution. Solid parts of homogenate were removed by filtration through four layers of gauze. Filtrate was centrifuged at the operating speed of 12 000 r.p.m. for 10 minutes. After pellet percolation in TE solution (twice), 450 µl of TE (washing) solution along with 50 µl of SDS (5 M) solution, 50 µl of SDS (6.5%) solution, 20 µl of proteinase K (20 mg in 1 ml) were added to pellet and it was incubated at 60°C overnight. After centrifugation at 10 000 r.p.m. operating speed for 5 minutes, 500 µl of phenol was added to the supernatant which was mixed up and left for 5 minutes and then again centrifuged at 10 000 r.p.m. speed for 5 minutes. Six-hundred µl of chloroform + isoamylalcohol (1:24) was added to water phase (in clean eppendorf test tube) and after mixing, the samples were left for a short time and then repeatedly centrifuged. This procedure (phenol+chloroform) was repeated twice. After addition of 96% ethanol to water phase in ratio of 1:2 the samples were left at -20°C for 10-12 hours. After centrifugation at 10 000 r.p.m. for 10 minutes, the sediment was dried out and dissolved in 20 µl of TE solution.

Conditions of PCR reaction

For amplification the following sequences of primers were used:

Enterococcus faecium,
direct: 5´ TAGAGACATTGAATATGCC 3´
reverse: 5´ TCGAAATGTGCTACAATC 3´
length of the fragment: 550 bp

Enterococcus faecalis,
direct: 5´ ATCAAGTACAGTTAGTTCT 3´
reverse: 5´ ACGATTCAAAGCTAACTG 3´
length of the fragment: 941 bp

PCR reaction was run in thermocycler MJ Research PT 150.

Temperature profile

The 1st cycle: 94 °C, 5 minutes.
Next 32 cycles: 94°C, 1 minute; 54°C, 1 minute; 72°C, 1.5 minutes.
Last cycle: 72°C, 10 minutes.
Amplified products were evaluated electrophoretically on 3% agar gel jelly and visualized under UV transluminator by means of ethidium bromide.

RESULTS AND DISCUSSION

The application of probiotic preparations based on *Enterococcus faecium* and prebiotic preparation based mannanoligosaccharides positively influenced caecal microflora of turkeys. Stavric and Kornegay (1995) also archieved positive results frequently in their trials with application of probiotic preparations.

The highest count of *Enterococcus sp*. log cfu in 1 g of cecal chymuse of 12 week-old turkey was detected in the treatment where prebiotic preparation based on mannanoligosacharides was added into feed at the quantity 2 $kg.t^{-1}$ (0,2 %). About the same counts were also found but few lower in treatments where preparation at concentration of 5.10^{10} was applied into water in graded amount by the age and weight from 2.10^8 to 30.10^8KTJ. g^{-1}. The lowest count of log $cfu.g^{-1}$ was recorded in the control treatment (Fig. 1).

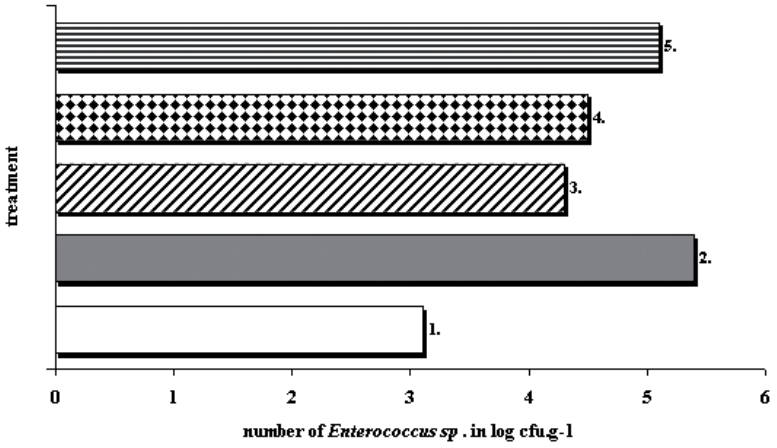

Fig. 1: Numer of *Enterococcus sp.* in log cfu.g^{-1}

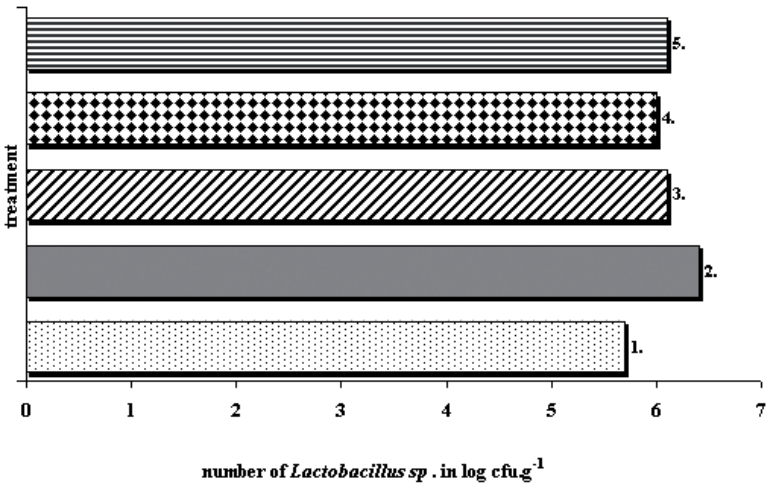

Fig. 2: Numer of cells of *Lactobacillus sp.* in log cfu.g^{-1}

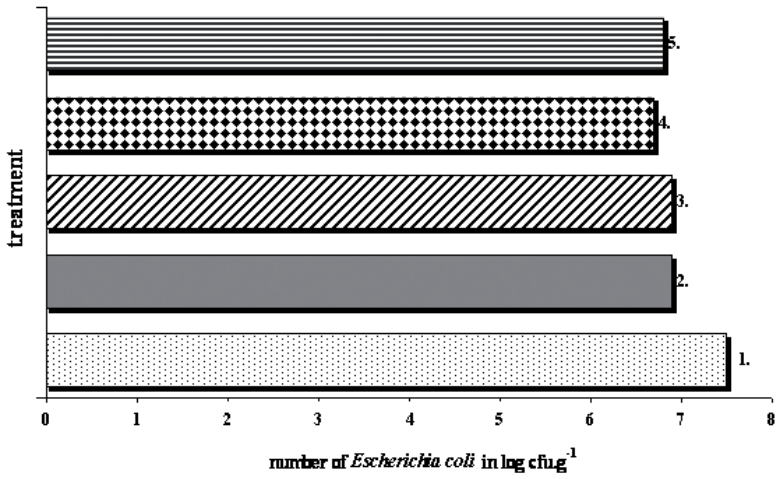

Fig. 3: Numer of Escherichia coli in log cfu.g^{-1}

The highest count of *Lactobacillus sp.* log cfu in 1 g of caecal chymuse of 12 week-old turkey was detected in the treatment where prebiotic preparation based on mannanoligosacharides were added into feed at the quantity of 2 kg.t^{-1} (0.2 %). About the same counts were also found in treatments where prebiotic preparation at the quantity 1 kg.g^{-1} (0,1 %) were applied into feed and probiotic preparation at the concentration of 5.10^{10} cfu.g^{-1} into water in graded amount by the age and weight from 2.10^{8} to 30.10^{8}KTJ.g^{-1}. The lowest counts of Lactobacillus sp. were recorded in the control treatment (Fig. 2).

Among 12 week-old turkey, the highest *Escherichia coli* log colony forming units (cfu) were detected in the control sample. All experimental treatments yield lower counts when compared to the control sample. However, the lowest count (Fig. 3) was found in the treatment where probiotic preparation was applied into feed daily followed by the treatments with application of *Enterococcus faecium* at the concentration of 5.10^{10}.

Enzymatic activity of cellulases in caeca of 12 week-old turkey was the most intensive in the treatment where probiotic preparation was applied into the water in graded amount by the age and weight. It was twice as high as in the control sample. When probiotic preparations were applied into feed and prebiotics, higher values of enzymatic activity were found, compared to the control (Fig. 4).

In general, probiotic preparation based on *Enterococcus faecium* positively influenced microflora of turkey caeca, an observation also confirmed by the results obtained by Kačániová et al. (2005).

The effect of probiotics is derived from their capability to reduce the counts of undesired microorganisms in the digestive tract mucous membranes. Thus, the host organism need not be focused to a permanent influence of unwanted microorganisms present directly in the oral, nosal and intestinal

mucous membranes. The role of protective microflora (Kačániová, 2001) is irreplaceable in relation to general well-being of animals and achievement of optimum weight gains.

The presence of *Enterococcus faecium* (applied into digestive system of poultry with the help of probiotic preparation) in caeca of chicken and turkey was detected by means of the polymerase chain reaction (PCR). The amplification gene of *Enterococcus faecium* in the polymerase chain reaction produced 550 bp fragment and that *Enterococcus faecalis* 941 bp PCR product.

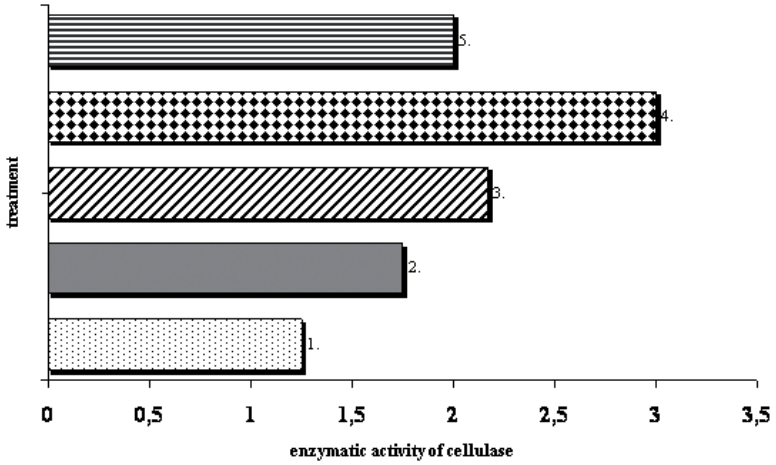

Fig. 4: Enzymatic activity of cellulases in CMC units

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