SPECIFIC FEATURES OF GASTROINTESTINAL TRACT MICROBIOCENOSIS IN HENS AND GEESE

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Abstract
The main objective of the present study was to determine the characteristics of microflora composition of caecum and colon in hens and geese reared under standard conditions. Some similarities and differences in microflora composition in some parts of the gastrointestinal tract of birds were shown. The maximum total amount of microorganisms was determined in the layer hens’ gastrointestinal tract, especially in the caecum content. Different groups of microorganisms decreased in the following order in hens: bifidobacteria, Lactobacillus, Enterococcus, Streptococcus, Staphylococcus and fungi. A different order was typical of geese: bifidobacteria, E. coli, Lactobacillus, Streptococcus, Staphylococcus and fungi. The intestinal tracts of geese were characterized by the presence of moulds.

Key words: microflora, caecum, colon, hen, goose

The natural microflora of animal organisms is the open biocenosis of microorganisms, which a large number of microbes enter with fodder. It is autoregulated by competition for substrates, places of adhesion and other factors and kept in equilibrium (Rolfe, 1984).

The symbiotic microflora of animals is necessary for vital activity of macroorganisms. It performs several specific functions such as supporting colonization resistance, forming immune response, synthesis and detoxification functions, and taking part in the process of metabolism (Gebbers and Laissue, 1984; Bordello, 1984).

Since the 1960s and 1970s, scientists have underscored the important role of gastrointestinal tract microflora of animals in the digestion and assimilation of food components (Bandaru et al., 1969; Furuse et al., 1992) and qualitative and quantitative composition of birds’ microbiocenosis (Salanitro et al., 1974). However, attempts to correct and influence its composition have been made since antibiotics were first used in poultry. This has led to disturbances in intestinal microecology.
When considering different methods of controlling avian infections, attention is paid mainly to eradication of pathogenic intestinal bacteria but not to the ways of supporting protective functions of natural bacterial flora. A positive effect of probiotics (Ayasan et al., 2006; Ahmad, 2006; Gerasimenko, 2005), prebiotics (Yaghobfar et al., 2006) and symbiotics (Corrier et al., 1992) on restoration of microflora composition and prevention of its disturbances in farmed birds has been achieved. However, in order to find disturbances it is necessary to know physiological microflora composition in different types of birds. High-quality composition of basic representatives of intestinal microbiocenosis does not differ with various species of birds, while quantitative differences are obviously present. In addition, it can also be influenced by bird diets, age, stress and other environmental factors (Scupham, 2007).

The main objective of the present study was to determine the characteristics of microflora composition of caecum and colon in various species of birds reared under standard conditions.

**Material and methods**

The samples (caecum and colon content) were taken from Shaver 579 laying hens aged 210 days and White Italian geese aged 2 years. The hens were housed in cages. The housing conditions were compatible with the existing technological requirements. Geese were given free access to food and water throughout the experiment. All birds were on balanced standard nutrition (Table 1).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Laying hens content (%)</th>
<th>Geese content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>40</td>
<td>20.5</td>
</tr>
<tr>
<td>Wheat</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>27*</td>
<td>25*</td>
</tr>
<tr>
<td>Oats</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Extruded peas</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Sunflower cake</td>
<td>9</td>
<td>3.6</td>
</tr>
<tr>
<td>Fodder yeast</td>
<td>4.5</td>
<td>2</td>
</tr>
<tr>
<td>Fish meal</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Meat-and-bone meal</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Wheat bran</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Chalk</td>
<td>6.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Defluorinated phosphate</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Feed fat</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Table salt</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Content in 100 g of mix feed (%)**

| Metabolizable energy (MJ) | 1.18 | 1.07 |
| Crude protein            | 17.2 | 14.6 |

*Naked barley.*
At the end of the experiment, all animals were killed and their intestinal tract was separated immediately after death. The samples of caecum and colon rectum contents (1 g) were transferred under aseptic conditions and diluted with saline solution (1:10) for determination of specific quantitative and high-quality microflora composition (Krasnogolovez, 1989). After dilution, 100 μl of each sample was planted onto the following media: Endo-agar (DDPBZ, Ukraine) for *E. coli*, Yolk-salt-agar (IBT, Ukraine) for *Staphylococcus*, MPA-agar (IBT, Ukraine) for *Streptococcus*, Vismut sulfite agar (Mikrogen, Ukraine) and Ploskirev agar (Mikrogen, Ukraine) for *Salmonella* and *Shigella* spp. Sabouraud-agar (Albatim, Ukraine) for fungi, Blaurokk-media (IBT, Ukraine) for bifidobacteria and *Lactobacillus* spp. These culture media were incubated at 37°C for 24–72 h. After the incubation the specific colonies on the selective culture media were counted and the numbers of viable colony forming units per g were calculated. The identification was conducted according to morphological, cultural, physiological and biochemical properties.

Statistical evaluation of the results was performed by Student’s t-test.

### Results

The results of the experiment showed that *Escherichia coli* strains with different fermentation activity ($10^6$–$10^7$ CFU/g) and *Enterococcus* ($10^4$–$10^5$ CFU/g) had the highest population level, except for bifidobacteria and *Lactobacillus*, in 1 g of caecum content (Figure 1). The correlation between *E. coli* strains with normal fermentation activity and lac(±)strains ranged from 84:16 to 93:7. Occasionally, the *E. coli* strains of hemolytics and lac(–) were separated. The total amount of coccus forms (*Strepto*- and *Staphylococcus*) did not exceed 10% of the total amount of microbes. *Candida* fungi and *Proteus* were shown in certain cases.

In the colon of hens, *E. coli* amounts ($10^5$ CFU/g) were less on exponent and enterococcus amounts were higher than in the caecum. However, we observed less *E. coli* normal fermentative strains (up to 56% of the total amount of microbes) in the hens’ colon than caecum. Some hemolytic *E. coli* strains, coccus forms, *Proteus* and fungi were present in the colon content, too.

Similar results were obtained for specific composition of the caeca and colon contents microflora in geese. They indicate larger amounts of microorganisms in caecum ($7.60 \log_{10}$CFU/g) than in colon ($4.57 \log_{10}$CFU/g, p<0.001) due to *E. coli* strains, bifidobacteria and *Lactobacillus* (Figure 2). The correlation between *E. coli* strains with normal fermentation activity and lac(±)strains was 97:4 in the goose caecum and 99:1 in the goose colon. Bifidobacteria count was higher than *Lactobacillus* count on 1.5–2.5 exponents (P<0.01) in both sections of the intestinal tract. The fungi, *Proteus*, *Strepto-* and *Staphylococcus* and moulds were present in the intestinal content, too.
The amount of caecum microflora was $10^6$–$10^9$ CFU/g but the amount of colon microflora was $10^5$–$10^7$ CFU/g in the experimental types of birds (laying hens, geese). The majority (about 99%) is formed by bifidobacteria and Lactobacillus strains. The rest is mainly formed by E. coli and Enterococcus. In addition, the total amount of Strepto- and Staphylococcus was $10^3$–$10^5$ CFU/g, and that of fungi was $10^2$–$10^4$ CFU/g in the caecum and colon of these types of birds. Proteus and hemolytic E. coli strains were noted in individual cases.
The interspecific microbiocenosis characteristics show that *E. coli* count was $10^6$–$10^7$ CFU/g in the caecum of hens and $10^7$–$10^8$ CFU/g in the caecum of geese, i.e. 10 times as much ($P<0.01$) (Figure 3). It was found that the amount of bifidobacteria and *Lactobacillus* strains, the basic microbiocenosis participants, was $10^{12}$ CFU/g in the caecum of hens. The *Lactobacillus* bacteria amount was only $10^6$–$10^7$ CFU/g and bifidobacteria amount was 109 CFU/g in the caecum content of geese. It was $10^5$ and $10^3$ times less ($P<0.01$) than these indexes in hens. It is worth mentioning that if the amount of bifidobacteria and *Lactobacillus* strains in the caecum contents of hens is identical, the *Lactobacillus* amount is three exponents less in the caecum of geese ($P<0.05$).
The total amount of microorganisms in the colon of geese ($4.6 \log_{10} \text{CFU/g}$) was two exponents less than in hens ($6.58 \log_{10} \text{CFU/g}$) ($P<0.01$), but no significant difference in the counts of *E. coli* was detected. The colon microflora composition of geese and hens is characterized by the same dependence on bifido- and *Lactobacillus* amount as in caecum. The bifidobacteria amount is $10^4$ times and the *Lactobacillus* amount $10^5$ times higher ($P<0.025$) in the colon of hens compared to geese (Figure 4). Also, the *Lactobacillus* amount in the colon of geese is approximately $10^2$ times less than the bifidobacteria amount ($P<0.01$).

**Discussion**

At the moment of hatching the gastrointestinal tract of nestlings is sterile and right after hatching it is being populated by environmental microorganisms. Young birds are more sensitive to colonization of pathogenic bacteria due to unformed intestinal microbiocenosis (Edwards and Parret, 2002). Therefore, a major problem of healthy poultry production is to provide for the rapid and valuable formation of microflora composition of the gastrointestinal tract in nestlings.

The natural microflora with its specific functions characterizes the gastrointestinal tract microbial ecology and takes part in the maintenance of macroorganism homeostasis (Pavlova et al., 2006). Every microorganism group in the intestinal tract performs its function. *Bifidus* bacteria generate organic acids and create unfavourable conditions for reproduction of pathogens, and produce vitamins B$_6$ and B$_{12}$ (Modler et al., 1990). Lactobacilli generate antibiotics, synthesize proteins, and ferment carbohydrates and alcohols (Bruno and Montville, 1993). *Escherichia coli*, being strict anaerobes, use oxygen and create favourable conditions for other bacteria, and secrete colicsins, which inhibit the growth of pathogenic microorganisms (Gokce and Lakey, 2003).

The composition of forage influences birds’ health and their resistance to pathogenic microflora. It is known that the microflora provides for complete degradation and assimilation of nutrients (Langhout, 1999; Riddel and Kong, 1992). The intestinal microbial associations are substrate-specific and therefore they depend on nutrient presence in the occupation zone. In birds on a diet with cellulose hypercontent (geese), researchers found higher amounts of cellulolytic microorganisms and other microbe groups, which utilize polysaccharides (for example, *Bacteroides*). The amount of amylolytic microorganisms, bacteria and *Streptococcus* increases with the increasing amount of dietary carbohydrates (Nikolicheva, 1978).

It is known that the basic microorganisms for animals or birds are facultative and strict anaerobe bifidobacteria, *Lactobacillus* and lactate-fermentation bacteria, and *Bacteroides* (Wise and Sirogusa, 2007; Salanitro et al., 1974; Yaghobfar et al., 2006). It has been detected that nearly 99% of the total amount of microorganisms in the caecum and colon of different bird types is made up by bifidobacteria and *Lactobacillus*. Facultative intestinal microflora is represented by opportunistic Staphylococci, Streptococci, hemolytic coli-forms, *Proteus* and fungi.
The maximum total amount of microorganisms was determined in the gastrointestinal tract of laying hens. Different groups of microorganisms decrease in the following order: bifidobacteria, *Lactobacillus*, *E. coli*, *Enterococcus*, *Streptococcus*, *Staphylococcus* and fungi. A different order was typical of geese: bifidobacteria, *E. coli*, *Lactobacillus*, *Streptococcus*, *Staphylococcus* and fungi. The intestinal tract content of geese was characterized by the presence of moulds. Ziółkowska and Tokarzewski (2007) have made the same observation. This difference is probably associated with the high cellulose content of the goose diet, with peculiarities of gastrointestinal tract structure and fodder energy utilization.

In general, we determined higher amounts of microorganisms in the birds’ caecum content than in colon. Our findings agree with the results reported for bacterial activity in some parts of the gastrointestinal tract of various bird species (Mul and Berry, 1994). In particular, Mul and Berry reported higher bacterial activity of caecum content, compared to colon, for hens and geese. It was shown that the bacterial activity of all intestinal tract parts was higher in geese than in hens. This concurs with our results. Similar results concerning the amount of organic acids in different parts of the intestinal tract of geese were obtained by Clemens et al. (1975).

The data obtained are the first stage of research to determine the characteristics of the composition of intestinal tract microflora in birds. The next step will be to explore the intestinal microbiocenosis composition of birds depending on feeding type, environmental factors and birds’ age. This will allow correcting unfavourable changes and improving nutrient digestion.

References

Specyficzne cechy mikrobiocenozy przewodu pokarmowego kur i gęsi

STRESZCZENIE


Marta Kamińska, Alla Huchach, Franciszek Borowiec, Irenej Ratych, Jan Barteczko