

THE COMPARATIVE ANALYSIS OF SOME REPRESENTATIVE BACTERIA OF BIRD GUT MICROBIOTA IN URBAN AND RURAL AREAS

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Abstract. Environmental changes significantly influence the health and behaviour of animals and determine their adaptation to new living conditions. Thus, urbanization, which is intensifying worldwide, represents new ecological and evolutionary challenges for animals. The state of the intestinal microbiota is an indicator that reflects the influence of environmental factors on organisms, as it is known that the intestinal microbiota mediates processes such as digestion, immunity and resistance to pathogens. Thus, in the conditions of urbanization and anthropization, new questions arise about how the urban environment shapes intestinal microbial communities and how the intestinal microbiome influences the survival of the host organism to the action of anthropogenic factors. The research on some representatives of compulsory and facultative bacteria in the intestinal content of birds in urban and rural environments highlighted the dominance of certain groups of bacteria in the intestinal content depending on the living environment. Thus, facultative bacteria (*E. coli*, enterococci) quantitatively predominate in the digestive tract of rural birds, while beneficial obligate bacteria (bifidobacteria and lactobacilli) predominate in the intestinal contents of urban birds. At the same time, in the gut microflora of urban birds, a positive correlation was established between enterococci, bifidobacteria and lactobacilli in keeping the number of *E. coli* at a lower level, which does not allow for the development of intestinal dysbiosis. The obtained data allow us to assume that the living environment, as well as the frequent and close contact of animals (in the given case of birds) with humans dictates the incidence of compulsory beneficial and facultative pathogenic bacteria of the intestinal microflora, which ultimately determines the health and well-being of the bodies.

Keywords: gut bacteria, rural and urban areas, birds.

Rezumat. Analiza comparativă a unor bacterii reprezentative ale microbiotei intestinale ale păsărilor din zonele urbane și rurale. Modificările mediului ambiant influențează semnificativ sănătatea și comportamentul animalelor, întrucât determină adaptarea lor la noile condiții de viață, iar urbanizarea, în acest context, reprezintă noi provocări ecologice și evolutive pentru animale. Starea microbiotei intestinale reprezintă un indicator care reflectă influența factorilor de mediu asupra organismelor, întrucât se cunoaște că microbiota intestinală mediază procese precum digestia, imunitatea și rezistența la agenți patogeni. Astfel, în condițiile urbanizării și antropizării, apar noi întrebări despre modul în care mediul urban modelează comunitățile microbiene intestinale și cum microbiomul intestinal influențează supraviețuirea organismului gazdă la acțiunea factorilor antropici. Cercetările unor reprezentanți ai bacteriilor obligative și facultative în conținutul intestinal al păsărilor din mediul urban și rural, a evidențiat dominanța anumitor grupe de bacterii în conținutul intestinal, în funcție de mediul de viață. Astfel, bacteriile facultative (*E. coli*, enterococii) predomină cantitativ în tractul digestiv al păsărilor din mediul rural, în timp ce bacteriile obligatorii benefice (bifidobacterii și lactobacili) predomină în conținutul intestinal al păsărilor urbane. Totodată, la păsările din mediul urban a fost stabilită o corelație pozitivă între enterococi, bifidobacterii și lactobacili, în menținerea la un nivel mai scăzut a numărului de *E. coli*, ceea ce nu permite dezvoltarea disbiozei intestinale. Datele obținute permit de a presupune că mediul de trai, precum și contactul frecvent și restrâns al animalelor (în cazul dat al păsărilor) cu oamenii, dictează incidența bacteriilor obligatorii benefice și a celor facultative patogene ale microflorei intestinale, ceea ce determină în final sănătatea și bunăstarea lor.

Cuvinte cheie: bacterii intestinale, mediul rural și urban, păsări.

INTRODUCTION

The processes of urbanization or anthropization of natural landscapes have led to changes in the adaptation strategies to natural phenomena, so that, since the rise of the industrial revolution, animals, plants and microbes have faced ecological pressure, which caused the reorganization of ecosystems and changes in biodiversity (LABBATE, 2016).

Many species of animals are forced to adapt to new conditions, and to change their foraging behaviour, in accordance with new food sources. In anthropized areas, in addition to food sources, there are other various stress factors that affect the health and fitness of the animals. Thus, urban habitats present new ecological and evolutionary challenges for animals (WINCHELL et al., 2022).

One potential factor reflecting the status of individuals in a species population is the status of the gut microbiome. The gut microbiota is known to play a fundamental role in host health and well-being, as it mediates processes such as digestion and nutrient synthesis, immunomodulation and resistance to pathogens, which can be shaped by host traits and environmental factors.

Today, urbanization is one of the most radical forms of land modification in terrestrial ecosystems, increasingly affecting the ecology of natural populations, including host-microbiota interactions, with visible differences observed in gut microbiota between urban and rural hosts (TEYSSIER et al., 2020).

Nutrition, development and immunity - all of which are affected by the gut microbiome - are important factors that can determine survival in urban environments. Therefore, ecologists are asking new questions about how the urban environment shapes gut microbial communities and how the gut microbiome affects host organism survival in an urban context (BERLOW, 2021).

Investigation of gut microbiota in different living (housing) environments has been carried out in several studies. Habitat urbanization is assumed to affect gut microbial composition and diversity, in particular through direct dietary and/or indirect environmental effects. TEYSSIER et al. (2020) showed that the gut microbiota in urban sparrows (*Passer domesticus* L.) is less diverse and has a different taxonomic composition and lower levels of seasonal variation than rural birds.

On the other hand, in another study conducted by PHILLIPS et al. (2018), it was determined that the microbiome of birds (white-crowned sparrow - *Zonotrichia leucophrys*, Foster) in urban and rural environments differs, and the urban gut microbiome is more diverse than the rural gut microbiome. The authors supposed that “this relationship may be due to more variable land cover types in urban habitats compared to rural habitats, which are composed mainly of native flora (scrub)” (PHILLIPS et al., 2018). The same authors later reported that noise in large cities, land cover peculiarities in urban and rural areas indirectly influence the alpha and beta diversity of gut microbial communities (BERLOW et al., 2020). It has been established that noise causes an increase in microbiome diversity, which is maintained at a higher level (compared to the control) after the action of the stressor has ceased (BERLOW et al., 2022).

In general, microbial communities reflect and are possibly influenced by heterogeneous environmental changes that are typical of urban space, and the choice of the framework used to define urban space may influence the results of studies investigating animal-microbe symbiosis in urban or anthropized conditions (MARACI et al., 2022).

Thus, the aim of the research was to reveal the microbiota status of urban and rural birds on the basis of changes in some gut bacteria, which would elucidate the potential function of microbial symbionts in the adaptation of the hosts to different environmental conditions, especially to anthropogenic factors.

MATERIAL AND METHODS

The investigations were carried out on four groups of birds: mallard (*Anas platyrhynchos* (Linnaeus, 1758) in urban areas and from captive conditions (Chisinau Zoo), domestic pigeons (*Columba livia* f. *domestica*) in urban and rural areas; pedigree pigeons (French curly breed) and Muscovy ducks in rural areas. The mallard is a wild species adapted to urban landscapes and to the captive condition of the Zoo. The pedigree pigeons and Muscovy ducks are domestic birds kept in individual households. *Columba livia* f. *domestica* are free-living pigeons in urban and rural environments.

For an analysis of the quantitative level of bifidobacteria, lactobacilli, enterococci and *Escherichia coli* in the intestinal contents, faecal samples in the amount of 1 g per were taken. Faecal samples are recognized as a useful alternative for the analysis of gut microbes and provide an appropriate evaluation of the latter (VIDEVALL et al., 2018, BERLOW et al., 2019, GARCIA-MAZCORRO et al., 2021).

In urban areas, bird faeces samples were collected from Chisinau Zoo, from the “Valea Morilor” Park and from an urban park square. In rural areas, the faeces matter of birds was collected from individual households. The samples were collected immediately after defecation in the amount of 1 g. From the place of collection to the laboratory, the samples were kept in a portable refrigerator, in order to avoid the denaturation of the microbiological material. The microbiological analysis was performed in the day of sample collection.

The content of microorganisms was determined using classical microbiological methods (GARMASHEVA & KOVALENKO, 2010). Their inoculation of microorganisms was performed on a culture medium specially prepared for each group of bacteria – bifidobacteria on Bifidobacterium Agar (produced and marketed by the company “Himedia”), lactobacilli on MRS Agar (“Himedia”), enterococci on Bile Esculine Azide Agar ISO 7899-2 (“Himedia”) and *Escherichia coli* on Endo Agar (“Himedia”). Each group of microorganisms were studied and counted separately.

In order to establish the number of viable microbial cells for every group of investigated microorganisms, the initial material (faecal sample), in the amount of 1 g, was taken for each determination. A series of dilutions were performed afterwards – diluting the intestinal content samples from 10⁻¹ to 10⁻⁹, then inoculating and incubating them on a nutrient medium, elective to each microorganism species. After incubation at (37±1) °C for 48-72 hours under aerobic or anaerobic conditions, depending on the microorganism species, typical colonies grown on Petri dishes with the last dilutions were counted. The number of viable bacteria in 1 g of intestinal contents is determined by the formula:

$X = a \cdot 10^n$, where

X - is the number of viable individuals in 1 g of intestinal contents;

a - the number of colonies in the last Petri dish in which growth is recorded;

n - is the degree of dilution.

Bacterial growth activity was determined by counting colony-forming units (CFU) on Petri dishes after successive dilutions. The number of CFU is expressed in decimal logarithms (lg). The viability of strains on experimental medium is expressed as % relative to control. Assays were performed in 3 replicates (SM EN ISO 4833:-2014).

The experiments were carried out in accordance with Directive 86/609 /EEC of 24 November 1986 on the Protection of Animals Used for Experimental and Other Scientific Purposes and were approved by the Methodical and Ethics Committees of the Institute of Physiology and Sanocreatology, Moldova State University.

RESULTS

The influence of ecological and anthropogenic factors on the gut microbiome has scarcely been investigated in natural populations. It is thought that frequent contact between humans and animals, especially in everyday life, can cause the mutual exchange of bacteria, including pathogens, which can have a negative impact on digestive tract health in both humans and animals.

This prompted us to analyse the incidence of some intestinal bacteria, whose level determines the health status of the digestive tract: bifidobacteria, lactobacilli as obligate intestinal bacteria, and enterococci, *E. coli* as facultative bacteria in intestinal content of animal species, the habitat of which differs, i.e. in rural areas where animals are cared for by humans and in urban areas where anthropogenic action is evident. The research was carried out on birds, as birds have different feeding habits and the ability to move long distances, being in contact with many different places and spaces (parks, gardens, lakes, forests). Often, the gut microflora of birds is perceived as an ecological indicator of the state of natural ecosystems (WAITE & TAYLOR, 2015; WU et al., 2021).

The comparative analysis of the numerical indices of bifidobacteria, lactobacteria, enterococci and escherichia, would allow to elucidate aspects of the influence of the human factor in rural and urban environments on intestinal microbiocenosis in animals, which in turn reflects the health status of the organism.

In urban areas, the investigations were taken from free-living pigeons and wild ducks; in rural areas, from free-living pigeons, pedigree pigeons (French curly breed) and Muscovy ducks. It should be noted that the pigeon in urban and rural areas is the domestic pigeon (*Columba livia* f. *domestica*), which is found near human settlements, in villages and in towns. The wild duck is the mallard or *Anas platyrhynchos* L. species, adapted to human settlements. In the urban environment, faeces samples were collected from mallards in the “Valea Morilor” Park and from the Chisinau Zoo (for comparative study) and faeces matter from pigeons - in urban park square. In rural areas, samples were collected from individual households (Fig. 1).



Figure 1. The living environment of mallard in urban (A) and of Muscovy ducks in rural (B) areas.

The obtained data reveal the variation of the investigated gut bacteria count depending on the living environment, and the results are reflected in Figs. 2 and 3.

The level of obligate beneficial bacteria (bifidobacterial and lactobacilli) and of facultative bacteria (enterococci and *Escherichia*) reflects the health state of the intestinal micro-biocenosis. As it can be seen from the data in Fig. 2, the bifidobacteria and lactobacilli indices are higher in the intestinal contents of birds in urban compared to rural and zoo conditions. Thus, the numerical value of bifidobacteria is 1.1 and 1.2 times higher compared to their content in domestic and zoo wild ducks respectively. The lactobacilli count in faecal matter of birds from the urban environment is 1.13 times higher in comparison with the same birds under captive conditions in the Zoo and 1.2 times higher compared to domestic ducks in rural zones.

Among the investigated bacteria, enterococci occupy a special position; as commensal bacteria, they influence the intestinal microbial balance and, hence, the health of the digestive tract. At the same time, these bacteria are agents of several intestinal infections. It has been established that the level of enterococci in the intestinal content of Muscovy ducks, from the rural environment, is about 1.4 times higher than the one of wild ducks - mallard in urban areas (Fig. 2). It is interesting to note that the numerical value of enterococci in the intestinal content of mallard from Chisinau Zoo is highest and constitutes 7.8 (lg/g), being close to the value (7.2 lg/g) established for birds in rural conditions (Fig. 2. A and

C.). The lowest value of enterococci was counted in the intestinal content of mallards in urban conditions that correlates with high values of beneficial (obligate) bacteria, recorded in the intestinal contents of birds in urban environments. Here, however, it is necessary to mention that the samples were collected from the “Valea Morilor” Park, where the habitat of the birds is similar to the natural one, including in terms of food sources (Fig. 1 A.). This also denotes the numerical value of *Escherichia* in the intestinal content of these birds, which is higher (1.4 times) compared to wild birds kept in captivity under controlled conditions, and lower (1.13 times) compared to domestic birds in individual rural households. It should be noted that the higher level of *Escherichia* bacteria suggests intestinal dysbiosis and respectively the body health state.

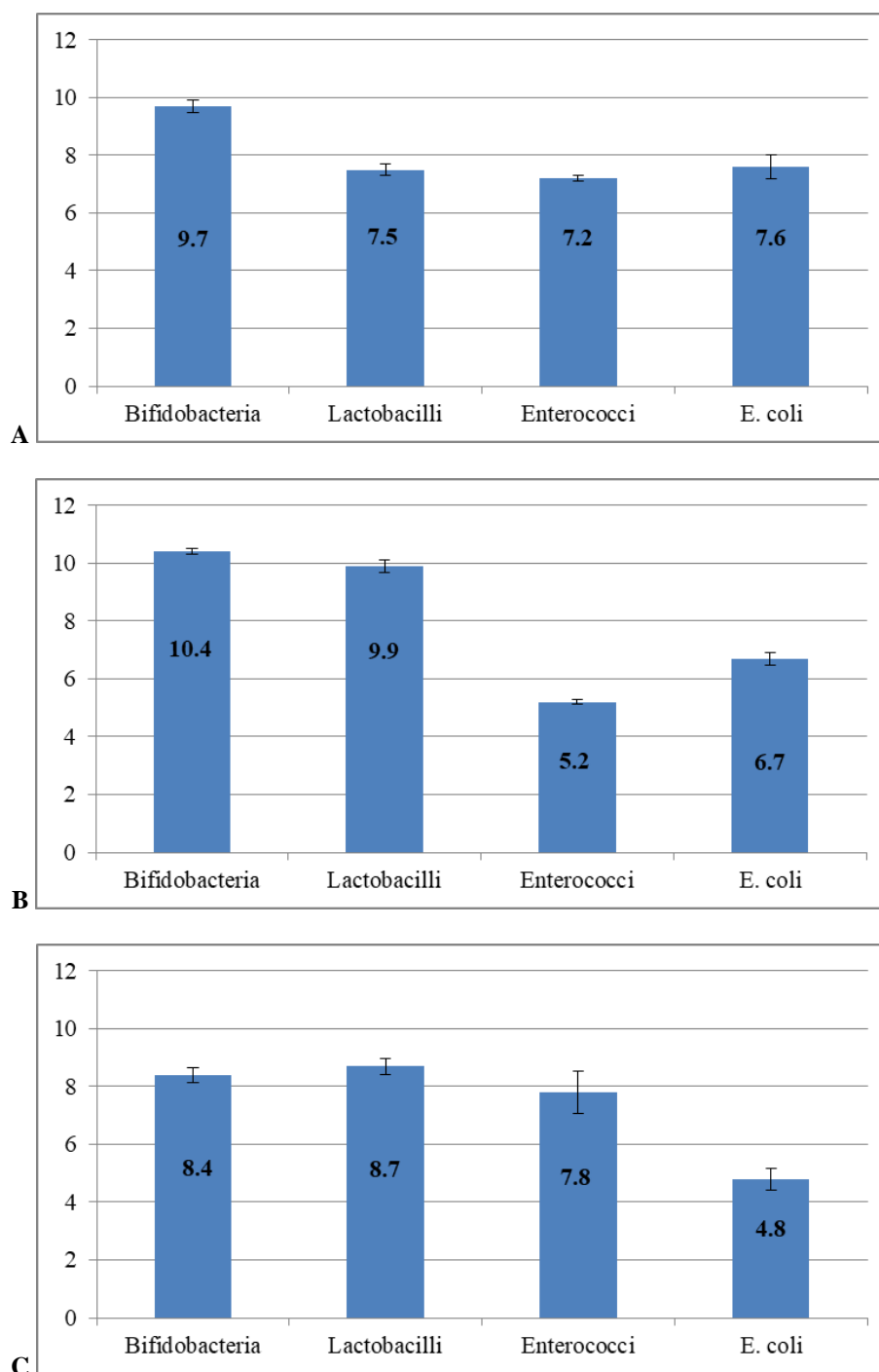


Figure 2. Quantitative levels of enterococci, bifidobacteria, lactobacilli and *Escherichia coli* in the intestinal contents of Muscovy ducks in rural environment (A) and mallard in urban zone (B) and zoo (C) conditions (expressed in lg/ 1 g of intestinal contents).

The same trend of numerical value changes in obligate and facultative bacteria was seen in the intestinal content of pigeons from rural and urban settlements (Fig. 3). An increased level of beneficial bacteria – bifidobacteria and lactobacilli (9.7 and 9.5 lg/g respectively) was established in the faecal matter of urban pigeons compared to the same birds (species) in rural areas (9,0 and 8.1 respectively). However, the levels of bifidobacteria and lactobacilli in the

intestinal content of free-living pigeons in urban areas are approximately the same as in French curly pigeons reared under special household conditions (9,7 and 9,5 lg/g vs 9.6 and 9.2 lg/g).

The evident dominance of enterococci was observed in the intestinal content of pedigree pigeons, reared under domestic conditions, and in free-living pigeons (*Columba livia* f. domestica), both from rural areas compared to the same urban birds (*Columba livia* f. domestica) (Fig. 3.). Thus, the number of enterococci in the intestinal contents of pedigree pigeons (French curly) was 6.2 lg/g, being about 1.12 times higher than the content of these bacteria in faeces samples of rural free-living pigeons, and about 1.3 times higher than of urban free-living pigeons. The level of pathogenic bacteria (*E. coli*) is also higher in faeces samples of rural birds (about 1.4 and 1.5 times) than that of urban birds (Fig. 3).

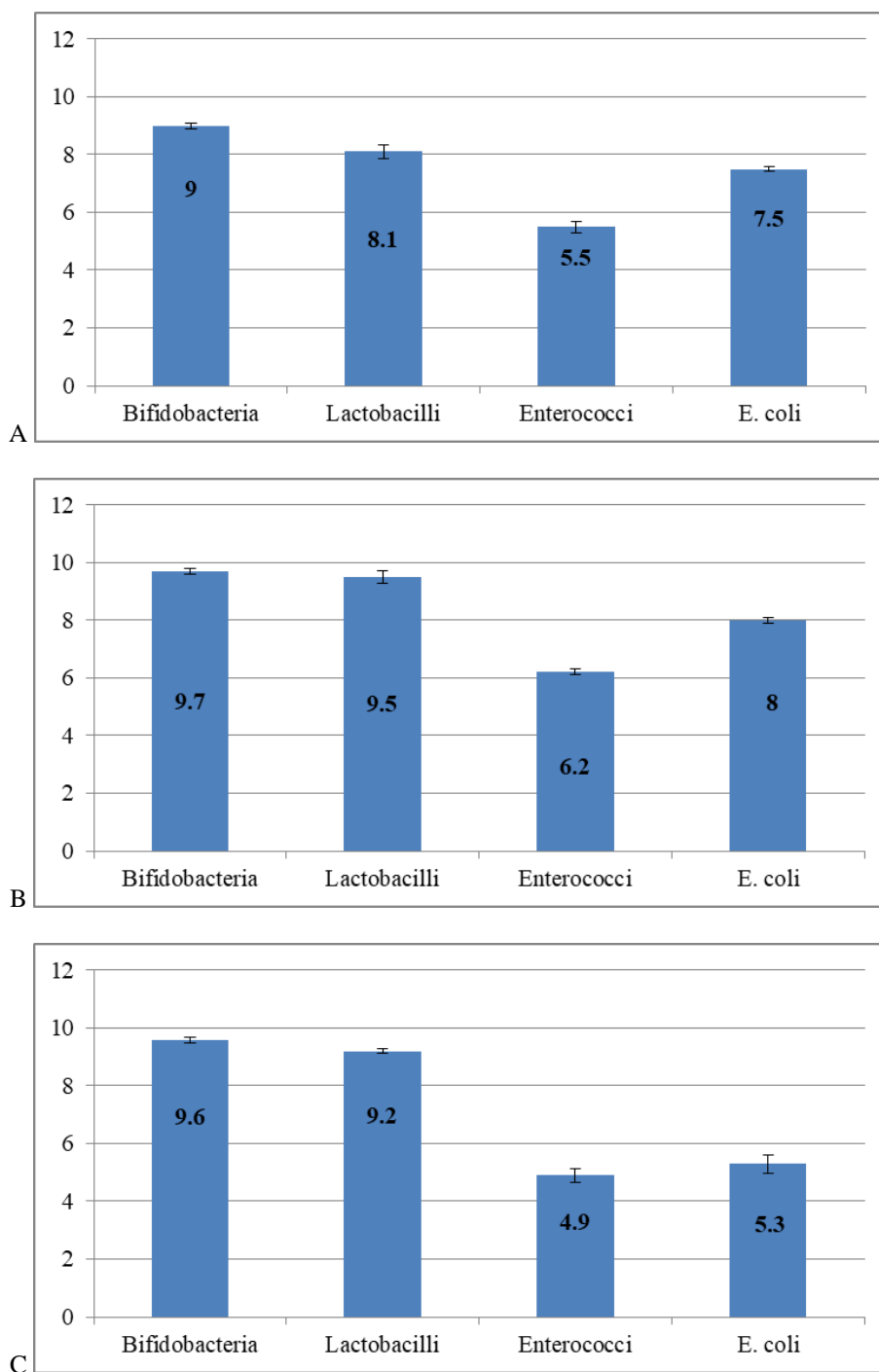


Figure 3. Quantitative levels of enterococci, bifidobacteria, lactobacilli and *Escherichia coli* in the intestinal contents of free-living pigeon in rural area (A) of pedigree pigeons - French curly in rural individual households' conditions (B) and free-living pigeon in urban area (C) (expressed in lg/ 1 g of intestinal contents).

Analysing the values of beneficial bacteria in comparison with those of enterococci and *E. coli*, it is observed that the intestinal bacteriocenosis status of rural birds is less favourable than that of urban and zoo birds. This indicates

that the digestive tract health of birds and other animals in rural areas is exposed to more risk factors. The higher level of facultative bacteria in gut samples of rural birds may be due to permanent contact with humans (with certain people) or with other animals as an additional source of bacteria, indicating the presence of infection outbreaks in the rural environment, which can affect the composition of the gut microflora.

The fact that humans can be a source of bacteria for the animals they come into contact with is also reflected by data on the *Escherichia* count in the faecal matter of rural birds, especially in the pedigree pigeon, where the numerical value is about 1.3 times higher than in samples of urban pigeons.

In urban birds, there is a more positive correlation between enterococci, bifidobacteria and lactobacilli in keeping the number of *E. coli* at a lower level. Thus, the numerical values of bifidobacteria in the intestinal content of urban birds is about 2 times higher than those of enterococci and about 1.6-1.8 times higher than those of *Escherichia*, and that of lactobacilli is about 1.9 times higher than that of enterococci and 1.5-1.7 times higher than that of *E. coli* (Figs. 2, 3).

In rural areas, this difference is smaller. The numerical index of bifidobacteria is 1.4-1.6 times higher than that of enterococci and 1.2-1.3 times higher than that of *Escherichia*, while that of lactobacilli is 1-1.5 times higher than that of enterococci and only 1.2 times higher than that of *Escherichia* (in the pedigree pigeon). In the domestic duck (Muscovy ducks), the level of lactobacilli was lower compared to the level of *E. coli* (Figs. 2, 3).

Thus, it was established that certain groups of bacteria predominate depending on the living environment: in the digestive tract of rural birds a high level of pathogenic (*E. coli*) and potentially pathogenic (enterococci) bacteria was found, while in urban birds, the one of beneficial bacteria – bifidobacteria and lactobacilli prevailed.

DISCUSSIONS

The incidence of certain intestinal bacteria in relation to the living environment is reflected quite extensively in the specialized literature. For example, the causes of higher numbers of certain bacteria in rural areas may be different and largely depend on the environment from which animals acquire their feed. The digestive tract can host representative samples of microbial communities present in the environment (SULLAM et al., 2012), resulting in local variations in gut bacterial communities (KLOMP et al., 2008).

Bacterial communities in water and soil have been shown to vary with the urbanization degree (WANG et al., 2017), and the gut microbiota composition of animals living in these places takes on site-specific traits.

In birds, passive uptake of microbiota present in the environment can take place, for example, through feather preening; correlations between pen and gut microbiota have been demonstrated in two bird species (van VEELLEN et al., 2017).

Thus, environmental factors are one of the main modulators of the gut microbiota as they influence the interactions between gut microorganisms as well as their interactions with their hosts. Also, these factors, faced by animals in their either natural or anthropogenic habitat, can cause a large imbalance of different types of gut microorganisms, inducing various digestive problems (ANWAR et al., 2021).

Another major mechanism that determines the composition and condition of the gut microbiota is the diet or way of nutrition of the hosts, which depends on both the living environment and host-specific traits such as nutritional status, food preferences or behaviour.

Food sources, most likely, are associated with certain bacteria and therefore play an important role in shaping the gut microbiota (WU et al., 2011). Several studies have shown differences in gut microbiota between hosts specializing on different food resources (e.g. in birds) (BODAWATTA et al., 2018).

Dietary changes in urban populations have been widely observed in mammals and birds (MURRAY et al., 2018). These are driven by the overabundance of non-native plants (in parks and dendrites) and food waste, which alters dietary requirements and/or feeding strategies (LIKER et al., 2008) and, as a result, can influence the status of gut microbiota (TEYSSIER et al., 2020).

On the other hand, feed resources under captive conditions can have a significant impact on the avian gut microbiota. The simple gut microbiota composition of captive or domesticated birds fed an immobilized diet has a poor stress response to external stimuli, resulting in a significant increase in the abundance of pathogenic groups in the gut bacterial community (CHEN & LI, 2022).

The high incidence of pathogenic bacteria in rural birds and poultry may be due to rearing conditions and the possible presence of infection outbreaks as a result of restricted contact with humans and other domestic animals (CHEN & LI, 2022), as well as the use of antibiotics in livestock and poultry husbandry (SUN et al., 2022).

Most likely, the living conditions, the mode of nutrition and the diversity of nutrients in the food ration of some animals, determines the over- or under-presence of certain obligate and facultative bacteria in the gastrointestinal tract. As a result, the composition of the microbiota, in relation with above mentioned factors, can reflect the health status of the host organism as well as how animals are kept.

CONCLUSIONS

It has been established that certain groups of bacteria prevail in the intestinal content depending on the living environment: facultative bacteria (*E. coli*, enterococci) predominate quantitatively in the digestive tract of rural birds, while beneficial obligate bacteria (bifidobacteria and lactobacilli) predominate in gut content of urban birds.

In urban birds, a more positive correlation between enterococci, bifidobacteria and lactobacilli was attested in keeping the number of *E. coli* at a lower level, which does not allow the development of intestinal dysbiosis.

The incidence of beneficial (obligate) and pathogenic (facultative) bacteria varies in different groups of birds depends to a large extent on their living environment and is significantly influenced by frequent contact with humans or other animals and by the action of anthropogenic factors.

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