THE PECULIARITIES OF THE COLONIZATION OF THE BIRDS' DIGESTIVE TRACT BY SOME REPRESENTATIVES OF INTESTINAL MICROBIOTA

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Abstract. The specificity of the digestive tract colonization by three cultures of bacteria specific to the intestinal microflora of birds: lactic acid bacteria (*Lactobacillus salivarius*, var. avius), bifidobacteria (*Bifidobacterium thermophilum*, var. avium) and *Escherichia coli*, M 17, on the model of gnotobiotic chickens was studied. Especially, the particularities of the interaction between the investigated bacteria, separately and in combination, in the digestive tract of germ-free chickens were determined. The interrelationships between bacteria in the digestive tract differ in the conditions of colonization with two and three types of bacteria. Thus, the combination of three types of bacteria positively influenced the multiplication of bifidobacteria in the crop and gizzard and of lactobacilli in the other segments of the digestive tract, as well as inhibiting the development of *Escherichia coli* in all segments of the gastrointestinal tract of birds. In the variants, in which the combinations of two species of bacteria were tested, the peculiarities of the interrelationships were different: the inhibition of the bifidobacteria multiplication in crop and gizzard either by of *Escherichia coli* or by *Lactobacillus salivarius*, var. avius actions, and the stimulation of the *E. coli* multiplication in ceacum by *L. salivarius*, var. avius action. However, the specificity of the colonization and development of the studied bacterial cultures depends not only on the ecological conditions and metabolic functions of the digestive tract compartments, but also on direct interactions between the bacterial species. The investigations also are important in the aspect of probiotic preparations development from the perspective of microbiota restoring through the colonization of the gut with representatives of the compulsory microflora.

Keywords: colonization, digestive tract, bifidobacteria, lactobacilli, Escherichia.

Rezumat. Particularităţile colonizării tractului digestiv la păsări de către unii reprezentanţi ai microbiotei intestinale. A fost studiat specificul colonizării tubului digestiv pe modelul puilor gnotobiotici la administrarea perorală a trei culturi de bacterii caracteristice microbiotei păsărilor: bacterii acidolactice (*Lactobacillus salivarius*, var. avius), bifidobacterii (*Bifidobacterium thermophilum*, var. avium) şi *Escherichia coli*, M 17. Au fost determinate particularităţile interacţiunii dintre bacteriile investigate, separat şi în combinaţie, în tractul digestiv la puii non-microbiali. S-a stabilit că interrelaţiile dintre bacterii în tubul digestiv diferă în condiţiile colonizării cu două şi trei tipuri de bacterii. Astfel, combinaţia a trei tipuri de bacterii a influenţat pozitiv multiplicarea bifidobacteriilor în guşă şi stomac şi a lactobacililor în celelalte segmente ale tractului digestiv, precum şi inhibarea dezvoltării *Escherichia coli* în toate segmentele tractului gastrointestinal la păsări. În variantele în care au fost testate combinaţiile a câte două specii de bacterii, specificul interrelaţiilor a fost diferit: inhibarea multiplicării bifidobacteriilor în guşă şi stomac, în cazul combinaţiei *Bifidobacterium thermophilum*, var. avium fie cu *Escherichia coli*, fie cu *Lactobacillus salivarius*, var. avius; stimularea dezvoltării *Escherichia coli* în ceacum, în combinaţie cu *Lactobacillus salivarius*, var. avius. Prin urmare, s-a stabilit, că specificul colonizării şi dezvoltării culturilor de bacterii studiate depinde nu numai de condiţiile ecologice şi de funcţiile metabolice ale compartimentelor tractului digestiv, dar şi de interacţiunile nemijlocite dintre speciile de bacterii. Investigaţiile efectuate, de asemenea, prezintă interes şi în aspectul elaborării de preparate probiotice, din perspectiva restabilirii microflorei prin colonizarea tubului digestiv cu reprezentanţii microflorei obligative.

Cuvinte cheie: colonizare, tract digestiv, bifidobacterii, lactobacili, Escherichia.

INTRODUCTION

The studies of the animals' intestinal microbiota are actually performed not only in terms of the health of the host organism, but also in terms of the ecology and biodiversity of host species populations. The changes in the gastrointestinal tract (GIT) microbial community significantly affect various aspects of the animals' life, such as nutrition, reproduction, and health (KOHL, 2012; SHANG et al., 2018).

On the other hand, external factors and the age and species characteristics of the host organism influence the composition and diversity of the intestinal microbiota. It is known that the intestinal microbiota itself can be interpreted as a component that characterizes the biocenotic diversity, representing an indicator of ecosystem stability. The bacterial community of the digestive tract itself represents a complex ecosystem, comprises about 50 genera and several hundred and even thousands of species (ECKBURG et al. 2005; FRANK et al., 2007).

The interest regarding studies of microbiota relationships with the host organism in the biocenotic aspect is also conditioned by the fact that the ecological principles that are applied to understand intra- and interspecific relationships in an ecosystem can be used to study host-microbe interactions and specific gut microbial functions (LOZUPONE et al., 2012).

At the same time, the composition, diversity and function of intestinal microbial communities could provide information on biodiversity conservation strategies.

Various research in animal biology has established that the composition and quantitative level of intestinal bacteria significantly influence the animal's life: the ability to resist the stressors' action, the inter- and intra-population behaviour of animals, communication, etc. (CRYAN & DINAN, 2012). An important aspect, in this sense, is the study of the peculiarities of a healthy intestinal microflora, especially in birds. Scientific research on these aspects also derives from the specificity of the colonization of bird digestive tract, which takes place in a different way from that occurring

in mammals and depending ratheron external factors (environment) (CARMEN COLLADO et al., 2016). The colonization of various segments of the digestive tract of animals, and the already established bacterial composition can serve as an indicator of the microbiota's good health, and the health of the body as a whole. The specificity of colonization depends on the species range, climate and seasonal changes, especially in the case of migratory birds. (CAREY & ASSADI-PORTER, 2017; SKEEN et al., 2020). Most experimental studies on the birds' intestinal microbiota have been performed on birds of economic interest (chickens, turkeys) (TIMOSHCO et al., 1979; GROND et al., 2018). It has been established that the microbiocenosis of gastrointestinal tract compartments in chickens is dominated by bacteria, followed by fungi, archaea, protozoa, and virus (WEI et al., 2013). Each type of bacteria is adapted to the internal ecological niche and is in synergistic relationships with other species of bacteria in the same community (OAKLEY et al., 2014).

The specific composition of the microflora in the digestive tract compartments also depends on their metabolic functions, not only on the specifics of the diet and habitation. A great variety of bacterial composition in the digestive tract has been established in birds fed with the same food (SHANG et al., 2018; CHOI et al., 2014).

Thus, the aim of the study was to reveal some peculiarities of the process of chicken's digestive tract colonization by the most commonly found representatives of the bird intestinal bacteria – cultures (strains) of *Lactobacillus salivarius*, var. avius, *Bifidobacterium thermophilum*, var. avium and *Escherichia coli,* M 17. These investigations will allow elucidating the specific interaction between microorganisms and the obtained date can be used in the development of probiotics for the poultry industry.

MATERIAL AND METHODS

The gnotobiotic chickens, raised in special conditions for gnotobiotic research, served as the object of study. The research was divided into three experiments. The bacterial strains – *Bifidobacterium thermophilum*, var. avium, *Lactobacillus salivarius*, var. avius and *Escherichia coli*, M 17 were obtained from domestic birds.

In the first experiment, the process of colonization of the digestive tract in gnotobiotic chickens by *Bifidobacterium thermophilum*, var. avium apart and in combination with *Escherichia coli*, M 17 was studied. For this purpose, the 3-day-old gnotobiotic chickens (no. 8) were fed orally with pure culture of *Bifidobacterium thermophilum*, var. avium in the amount of 1 million cells per chicken. 3 days later, the 6-day-old chickens were divided into two groups, with four (4) animals in each. In group I the content of bifidobacteria in the GIT segments was determined. The chickens from group II received a culture of *Escherichia coli*, M 17 in the amount of 1 million cells per chicken in order to stabilize the relationship between these bacteria. 3 days later, the content of both bacteria in the GIT segments of experimental chickens was studied.

In the second experiment, the process of colonization of the digestive tract of gnotobiotic chickens by *Lactobacillus salivarius*, var. avius, apart and in combination with *Bifidobacterium thermophilum*, var. avium, on the one hand, and in combination with *Escherichia coli*, M 17, on the other hand, was studied. For this purpose, the 3-day-old gnotobiotic chickens (no. 12) were fed orally with pure culture of *Lactobacillus salivarius*, var. avius in the amount of 1 million cells per chicken. 3 days later, 6-day-old chickens were divided into three groups, with four (4) chickens in each. In group I, the lactobacilli content in the gastrointestinal tract segments was determined. In group II, the *Bifidobacterium thermophilum* culture, var. avium, was administered in the amount of 1 million cells per chicken. *Escherichia coli*, M17 culture was administered in group III of gnotobiotic chickens, in the amount of 1 million cells per chicken. In groups II and III, a study of the relationships between the bacteria in the process of colonization was proposed. After 3 days, the content of all types of bacteria in the GIT segments of experimental chickens was studied.

In experiment III, the peculiarities of the gnotobiotic chicken's digestive tract colonization by the combination of three cultures of bacteria: *Lactobacillus salivarius*, var. avius, *Bifidobacterium thermophilum*, var. avium and *Escherichia coli*, M 17, was investigated. For this purpose, the 3-day-old gnotobiotic chickens (no. 4) were fed with a combination of the mentioned bacterial cultures in the amount of 1 million cells per chickens. After 3 days, the content of lactobacilli, bifidobacteria and Escherichia in the GIT segments was determined.

The content of microorganisms was determined using classical microbiological methods (GARMASHEVA & KOVALENKO, 2010). Their inoculation of microorganisms was performed on agar culture medium, (produced and marketed by the company "Himedia"). Over 72 hours after incubation of the inoculated samples on Petri dishes at 37±1 $^{\circ}$C, quantitative indices of microorganisms were calculated at 1 g of gastrointestinal contents (by multiplying the number of colonies by diluting the sample). The final results are expressed in decimal logarithms (log) (GOST 30518-97, 2000).

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 the Ethics Committees of the Institute of Physiology and Sanocreatology.

REZULTS

The specificity of GIT segments colonizing was studied on the model of gnotobiotic chickens by the oral administration of three cultures of bacteria specific to the bird's microbiota: lactic acid bacteria (*Lactobacillus*

salivarius, var. avius), bifidobacteria (*Bifidobacterium thermophilum*, var. avium) and *Escherichia coli*, M 17. The interest in these investigations was also conditioned by the elucidation of the interactions between two and three species of bacteria administered in the digestive tract of germ-free chickens. In fact, conditions of monomicroflora, dimicroflora and trimicroflora were created in the GIT of gnotobiotic chickens, i.e., in the conditions in which in the germ-free birds' digestive tract was only one, two or three cultures of bacteria.

The composition of the intestinal microbiota in birds is quite diverse, but certain groups of microorganisms have a stable presence and bifidobacteria are attributed to this group of bacteria. The obtained data revealed that in monomicroflora conditions, in 6-day-old gnotobiotic chickens, bifidobacteria easily colonize all compartments of the intestinal tract, except the crop. At the same time, it was established that the maximum content of bifidobacteria is attested in the rectum (12.6 log/g bacterial cells) and the minimum in the gizzard (3.86 log/g bacterial cells). As it was mentioned, these bacteria were not found in the crop (Table 1, Fig. 1).

Table 1. The average content of *Bifidobacterium thermophilum*, var. avium (log/g bacterial cells) in digestive tract segments of gnotobiotic chickens.

GIT segment	The average bacterial content (log/g bacterial cells)	Confidence interval, at P=95%
Crop	-	-
Gizzard	3.88 ± 0.21	3,69-4.11
Duodenum	7.66 ± 0.37	7.29-8.03
Jejunum	7.43 ± 0.52	6.93-7.97
Ileum	7.9 ± 0.17	7.73-8.07
Ceacum	12.16 ± 0.93	11.23-13.09
Rectum	12.7 ± 1.16	11.54-13.86

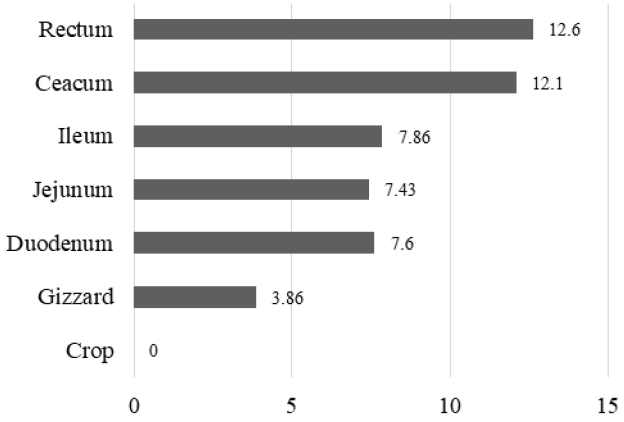

Figure 1. The *Bifidobacterium thermophilum*, var. avium content (log/g bacterial cells) in digestive tract segments of gnotobiotic chickens.

Afterwards, the 6 days-old chickens with bifidobacteria in the digestive tract, received an *Escherichia coli*, M 17 culture (the condition of dimicroflora was created). After 3 days, the dissemination of *Escherichia coli* was investigated, as well as the quantitative ratio of bifidobacteria and Escherichia, which reflects the interrelationships between these species of bacteria. The obtained data show that the bifidobacteria are not detected in the crop. The numerical value of Escherichia in this compartment is 2.5 log/ g of bacterial cells (Fig. 2). In the other GIT compartments, bifidobacteria predominate over the Escherichia (Fig. 2). In conditions of dimicroflora, the maximum content of bifidobacteria was found in the caecum, unlike to the monomicroflora conditions, in which their maximum content was attested in the rectum. It can be assumed that Escherichia has a stimulating effect on bifidobacteria multiplication. In general, the content of both bacteria increases from gizzard to rectum, although their quantitative ratio is not the same in GIT compartments. The biggest difference was established in the duodenum and caecum (Table 2, Fig. 2).

Table 2. The average bacterial content (log/g bacterial cells) in digestive tract segments of gnotobiotic chickens.

GIT segment	Types of bacteria	The average bacterial content (log/g bacterial cells)	Confidence interval, at P=95%
Crop	*B. thermophilum*	-	-
	E. coli	2.5±0.27	2.23-2.77
Gizzard	*B. thermophilum*	3.5±0.40	3.10-3.90
	E. coli	2.50±0.52	1.93-3.02
Duodenum	*B. thermophilum*	8.15±0.20	7.95-8.35
	E. coli	5.35±0.40	4.95-5.75
Jejunum	*B. thermophilum*	8.45±0.20	8.25-8.65
	E. coli	7.20±0.20	7.0-7.40
Ileum	*B. thermophilum*	10.32±0.19	10.13-10.51
	E. coli	7.80±0.13	7.67-7.93
Ceacum	*B. thermophilum*	12.55±0.33	12.22-12.83
	E. coli	9.02±0.75	8.27-8.77
Rectum	*B. thermophilum*	12.12±0.20	11.92-12.32
	E. coli	9.52±0.67	8.85-10.19

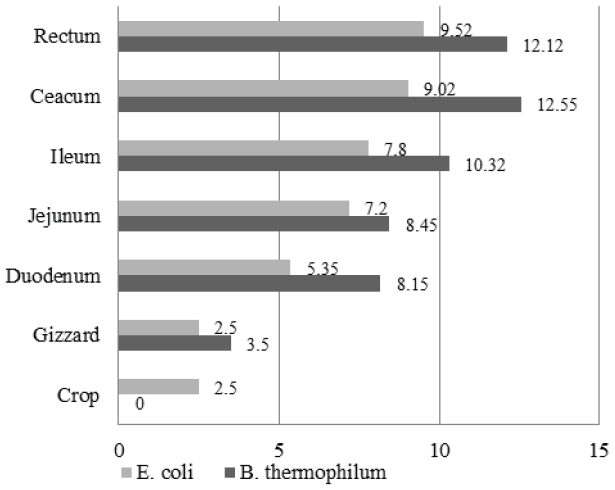

Figure 2. The content (log/g bacterial cells) of *Bifidobacterium thermophilum*, var. avium and *Escherchia coli*, M 17 in digestive tract segments of gnotobiotic chickens.

In the second experiment, the colonization of the digestive tract by the culture of lactobacilli was studied, and later the combination with the cultures of bifidobacteria and Escherichia, creating conditions of monomicroflora and dimicroflora respectively. Lactobacilli are of incontestable importance in the fight against dysmicrobism, as they show antagonistic action against pathogenic bacteria of the intestinal microbiota. Culture of *Lactobacillus salivarius*, var. avius was administered to 3-day-old chickens. After 3 days, the quantitative level of lactobacilli in the digestive tract compartments was determined. Lactobacilli have been found to be sown in large numbers (Fig. 3). It should be noted that unlike bifidobacteria, the representatives of this type of bacteria are present in all compartments of the digestive tract of gnotobiotic chickens. The minimum content was determined in the crop and gizzard (3.48 and 3.36 log/g of microbial cells), and the maximum – in the caecum and rectum, respectively 9.54 and 9.4 log/g of microbial cells (Table 3, Fig. 3).

Table 3. The average content of *Lactobacillus salivarius*, var. avius (log/g bacterial cells) in digestive tract segments of gnotobiotic chickens.

GIT segment	The average bacterial content (log/g bacterial cells)	Confidence interval, at P=95%
Crop	3.48±0.33	3.15-3.81
Gizzard	3.36 ± 0.34	3,02-3.70
Duodenum	5.50 ± 0.25	5.25-5.75
Jejunum	7.40 ± 0.20	7.20-7.60
Ileum	7.64 ± 0.34	7.30-7.98
Ceacum	9.54 ± 0.43	9.06-10.02
Rectum	9.40 ± 0.44	8.96-9.84

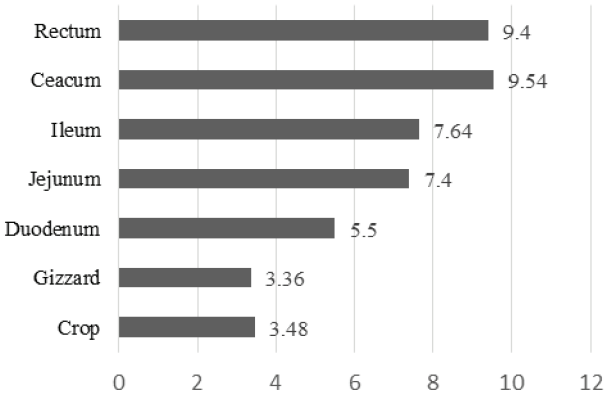

Figure 3. The *Lactobacillus salivarius*, var. avius content (log/g bacterial cells) in digestive tract segments of gnotobionts.

Upon inoculation (after 3 days) with bifidobacteria, it was established that lactic acid bacteria and bifidobacteria do not show reciprocal inhibitory action on their growth (Table 4, Fig. 4).

Table 4. The average bacterial content (log/g bacterial cells) in digestive tract segments of gnotobiotic chickens.

GIT segment	Types of bacteria	The average bacterial content (log/g bacterial cells)	Confidence interval, at P=95%
Crop	*L.salivarius*	3.75±0.79	2.97-4.55
	B. thermophilum	2.93±0.60	2.33-3.53
Gizzard	*L.salivarius*	3.13±0.37	2.76-3.50
	B. thermophilum	1.83±0.60	1.23-2.43
Duodenum	*L.salivarius*	8.00±0.50	7.50-8.50
	B. thermophilum	7.20±0.50	6.70-7.70
Jejunum	*L.salivarius*	7.80±0.89	9.91-8.69
	B. thermophilum	7.83±0.52	7.31-8.35
Ileum	*L.salivarius*	8.20±1.24	6.96-9.44
	B. thermophilum	9.00±0.50	8.50-9.50
Ceacum	*L.salivarius*	9.13±1.58	7.55-10.71
	B. thermophilum	11.30±0.93	10.37-12.23
Rectum	*L.salivarius*	9.25±0.77	8.49-10.03
	B. thermophilum	11.53±0.37	11.16-11.90

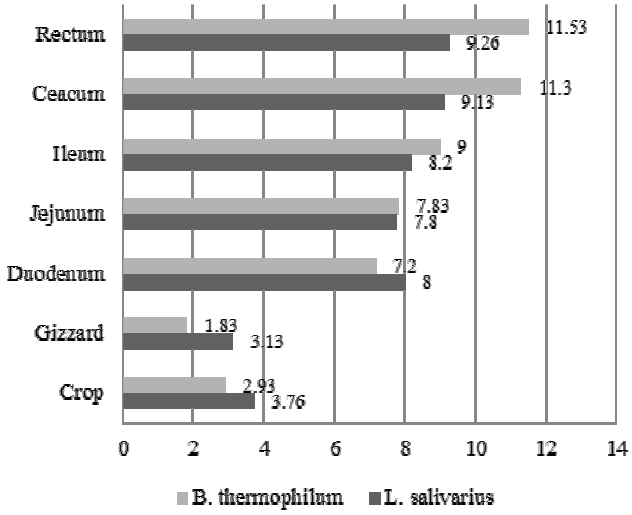

Figure 4. The content (log/g bacterial cells) of *Bifidobacterium thermophilum*, var. avium and *Lactobacillus salivarius*, var. avius in GIT segments of gnotobiotic chickens.

In the crop, gizzard and duodenum, lactobacilli predominated, and in another segment – bifidobacteria. The maximum content of lactobacilli and bifidobacteria was revealed in the rectum – 9.26 and 11.53 log/g of bacterial cells respectively. Thus, certain ecological relationships were established in the digestive tract of gnotobiotic chickens in conditions of dimicroflora (presence of two types of bacteria in gut), in which bifidobacteria prevails over lactobacilli. Lactobacilli, also, positively influence the multiplication of bifidobacteria in crop (Table 4, Fig. 4).

The interaction of lactic acid bacteria with *Escherichia coli* has also been studied on the model of gnotobiotic chickens. Escherichia was administered to 6-day-old chicks containing lactobacilli. That is, the conditions of dimicroflora – lactic acid bacteria and escherichia – were created. The determination of the quantitative level of the bacteria revealed that both *Lactobacillus salivarius* and *Escherichia coli* in the digestive tract of gnotobiotic chicks find

their comfortable niche for development (Fig. 5). This is also of practical importance, since the investigated bacteria has a certain beneficial effect on the functioning of the digestive tract and the whole macro-organism. Basically, lactobacilli predominate in the digestive tract, being in a larger amount in the rectum, caecum, ileum and jejunum. The *Escherichia coli* content is higher in caecum (8,92 log/g bacterial cells). *Lactobacillus salivarius* has been found to almost completely inhibit *Escherichia coli* in the gizzard (Table 5, Fig. 5).

Table 5. Average bacterial content (log/g bacterial cells) in digestive tract segments of gnotobiotic chickens.

GIT segment	Types of bacteria	The average bacterial content (log/g bacterial cells)	Confidence interval, at P=95%
Crop	*L.salivarius*	3.60±0.67	2.93-4.27
	E.coli	2.57±0.19	2.38-2.76
Gizzard	*L.salivarius*	3.37±0.27	3.10-3.64
	E.coli	-	-
Duodenum	*L.salivarius*	5.47±0.43	5.04-5.90
	E.coli	3.50±0.38	3.12-3.88
Jejunum	*L.salivarius*	7.55±0.40	7.15-7.95
	E.coli	6.55±0.62	5.93-7.17
Ileum	*L.salivarius*	7.65±0.62	7.03-8.27
	E.coli	6.72±0.64	6.08-7.36
Ceacum	*L.salivarius*	8.75±0.95	7.80-9.50
	E.coli	8.92±0.64	8.28-9.56
Rectum	*L.salivarius*	9.05±0.20	8.85-9.25
	E.coli	7.35±1.03	6.32-8.38

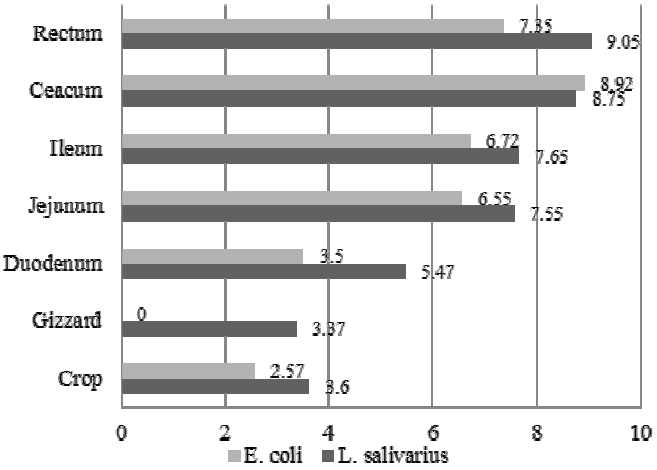

Figure 5. The content (log/g bacterial cells) of *Lactobacillus salivarius*, var. avius and *Escherchia coli*, M 17 in digestive tract segments of gnotobiotic chickens.

In experiment 3, the relationships between the three species of bacteria of the intestinal microflora in the GIT compartments of gnotobiotic chickens were investigated. Inoculation with the investigated bacterial cultures was performed on 3-day-old gnotobiotic chickens, and after 3 days the quantitative level of (log\g bacterial cells) was determined in the GIT compartments: crop, gizzard, duodenum, jejunum, ileum, caecum, rectum (Fig. 6).

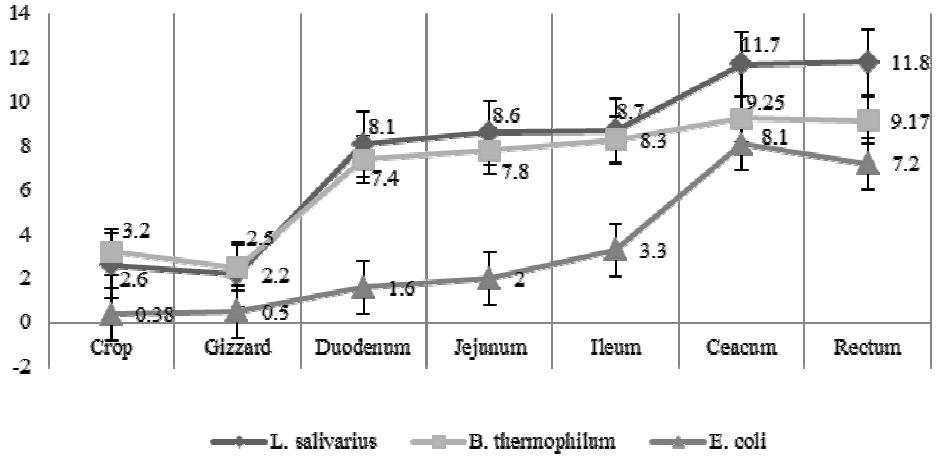

Figure 6. The content (log/g bacterial cells) of *Lactobacillus salivarius*, var. avius, *Bifidobacterium thermophilum*, var. avium and *Escherchia coli*, M 17 in GIT segments of gnotobiotic chickens.

The interrelationships between these species of bacteria differ from those of the combinations "bifidobacteria + escherichia", "lactobacilli + bifidobacteria" and "lactobacilli + escherichia". In general, in this combination all three cultures of bacteria grow normally in the digestive tract of the chickens.

Thus, in gnotobiotic chickens with trimicroflora, lactic acid bacteria predominate in the digestive tract, except for crop and gizzard, in which a higher amount of bifidobacteria was found. It should be noted that this combination of bacteria (trimicroflora condition) had a positive effect on the multiplication of bifidobacteria in the upper digestive tract, in comparison with monomicroflora (only bifidobacterial) and dimicroflora conditions (either "bifidobacteria + escherichia" or "lactobacilli + bifidobacteria"). In the other GIT compartments, the microflora was dominated by lactic acid bacteria (Fig. 6).

The combination of lactic acid bacteria and bifidobacteria inhibited the growth of *Escherichia coli* in all compartments of GIT of gnotobiotic chicks, although the nature of their content changes is the same as in the case of dimicroflora gnotobiotic chickens.

DISCUSSIONS

Recent research on intestinal microflora reveals an ecological balance between the host organism and bacteria and their relationships represent a symbiosis. Knowledge of the ecological structure of microbial systems can provide measures to control these systems by modulating the power and nature of the interactions between microbes and their environment (TROSVIK et al., 2010; BLAUT, 2013; COYTE et al., 2021). The micro-ecological interactions of the intestinal microbiota can be an indicator of the host organism state (KELLY et al., 2007). Most often the health of the organism correlates with the presence of bifidobacteria, lactic acid bacteria and Escherichia.

The obtained results regarding the content of some representatives of the bird's intestinal microflora in the digestive tract of gnotobiotic chickens demonstrated the data obtained in other studies. Thus, the lower content of the studied bacteria was detected in the crop and gizzard. Even at targeted administration of pure cultures of *Lactobacillus salivarius*, var. avius, *Bifidobacterium thermophilum*, var. avium and *Escherichia coli*, M 17 their quantitative value did not increase in these compartments (Fig. 6).

Each segment of the digestive tract is a niche for the development of certain types of bacteria, which are part of the intestinal microbiota. Both lactobacilli and bifidobacteria are beneficial and indigenous to the human and chicken GIT (REHMAN et al., 2007; WALTER, 2008). Because the main end product is lactic acid, lactobacilli prefer relatively acidic conditions (pH 5.5-6.5). That why the lactic acid bacteria predominate quantitatively in gizzard in comparison with bifidobacteria and Escherichia (Figs. 4; 5). Among the vast gut bacterial community, Bifidobacterium is a genus which dominates the intestine of most species or organisms, being in majority in the colon (RIVIÈRE et al., 2016). Escherichia are commonly found in the lower intestine (small intestine) of warm-blooded organisms (TENAILLON et al., 2010).

Indeed, the colonization process of the digestive tract is influenced by several internal host factors, such as pH, aerobic/anaerobic conditions, products of metabolism (food digestion), GI transit and consistency etc. Thus, the crop (with pH =5.5) is colonized predominantly by gram-positive bacteria such as Lactobacillus spp. Also, lactobacilli are the dominant species in the gizzard, which, with an acidic pH, is not ideal for microbial colonization. The conditions in the distal small intestine are favorable for microbial growth. The caecum conditions (with pH=8) contributed to the highest microbial density (FATHIMA et al., 2022). The diet and its nutritional content are a main factor that influenced the density and diversity of the gut microbiota, since the products digested in the GIT are used as a substrate in the activity (metabolism) of the bacteria (PAN & YU, 2014). It was also established that increased microbial diversity was related rather to longer descending colonic transit than to the consistency of intestinal content, because a longer distal colonic transit time may be accompanied by a greater and/or prolonged depletion of fermentable carbohydrates which in turn promotes diversification of bacteria (MÜLLER et al., 2020). Also, the oxygen availability influences the distribution of bacteria in the gut. The GIT is initially colonized by facultative aerobes such as Lactobacillus. Oxygen consumption by these bacteria facilitates subsequent growth and colonization of the extremely oxygen-sensitive obligate anaerobes in the lower gut. There are many other factors that influence GIT colonization by microorganisms. The elucidation of the relationships between the GIT microbiota and internal host environmental factors as well as dietary factors will constitute the goal of other more in-depth studies.

At the same time, it was established that the specificity of the colonization and growth of the studied bacterial cultures depends not only by the ecological conditions and metabolic functions of the GIT compartments, but also by the interactions between the investigated bacterial species. Recent findings show that the gut bacteria „communicate" via cell-to-cell signaling mechanisms, which determine the direct antagonistic and metabolic interactions and play a critical role in shaping the microbiota (THOMPSON et al., 2016). It should be noted that these interrelationships differ in the conditions of colonization with 2 and 3 types of bacteria. Certain relationships between the investigated bacteria are established under dimicroflora gut conditions of, and others under of trimicroflora gut conditions.

Thus, it was established that the multiplication of bifidobacteria in the crop and gizzard segments of GIT is stimulated by lactobacilli, especially in the combination of the three species of bacteria (Fig. 6). On the other hand, the association of three bacteria determined the intensification of the multiplication of lactobacilli in the intestinal compartments (duodenum, jejunum, ileum, clock, rectum), dominating quantitatively in these segments over bifidobacteria and Escherichia. At the same time, lactobacilli contributed to the increasing of Escherichia quantitative level of in the cecum, and in combination with bifidobacteria – to the inhibition of *Escherichia coli* multiplication in all compartments of the digestive tract, more obviously in crop and gizzard (Figs. 5; 6).

Therefore, gnotobiotic chicks can be used as an experimental model to study the process of adaptation/colonization of the digestive tract of animals by various species of microorganisms (representatives of the intestinal microflora). In the performed experiments, it was determined that the time required to determine the degree of adaptation of the microorganism strains in the digestive tract of gnotobiotic chicks should be not less than 3 days from the time of their oral administration. It is noteworthy that during the experiment the condition of the chickens was good, no cases of dismicrobism were detected. There were also no pathologies of internal organs, which indicates the positive effect of the tested strains of microorganisms.

CONCLUSIONS

It has been established that administrated monocultures of microorganisms – *Lactobacillus salivarius*, var. avius, *Bifidobacterium thermophilum*, var. avium and *Escherichia coli,* M 17, equally adapt in different segments of the digestive tract of gnotobiotic chicks.

The peculiarities of colonization and multiplication of the investigated bacteria depend not only on the functional (metabolic) specificity of the GIT compartments, but also on the interrelationships that are established between the bacteria in the dissemination process.

The stimulation of bifidobacteria multiplication in the upper segments of digestive tract (crop and gizzard) is conditioned by combined action of *Lactobacillus salivarius*, var. avius and *Escherichia coli*, M 17, and in the intestine segments (duodenum, jejunum, ileum, clock, rectum) by the unique action of either *L. salivarius*, var. avius or *E. coli*, M 17, i.e., in dimicroflora conditions.

The multiplication of *Lactobacillus salivarius*, var. avius in crop and gizzard is stimulated by the single action of *Escherichia coli*, M 17, and in the other gut segments by the combined action of *Bifidobacterium thermophilum* var. avium and *E. coli*, M 17 (under trimicroflora conditions).

The development of Escherichia is beneficially influenced by the separate action of *Lactobacillus salivarius*, var. avius. The combined action of *Lactobacillus salivarius*, var. avius and *Bifidobacterium thermophilum,* var. avium, results in the inhibition of Escherichia multiplication.

The investigations also are important in terms of probiotic preparations development from the perspective of microflora restoring by gut colonizing with the representatives of the obligatory microflora.

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