International Journal of Innovative Technology and Exploring Engineering (IJITEE)
ISSN: 2278-3075 (Online), Volume-9 Issue-1, November 2019

Method Development to Determine the Fractal Structures Index into the Broiler Chickens' Intestines

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Abstract: ***Microbial communities in the intestines of birds self-organize to carry out joint biochemical actions and extract nutrients from plant substrates. Depending on the feed entering the intestine, the configuration of the microbial biosystem is regularly reconstructed, evenly distributing the enzymatic load between the microbial components. To assess the qualitative and quantitative indicators of these processes, it is proposed to calculate the index of fractal structures of microbial communities existing in the intestines of birds. The technique is based on a fractal analysis of the frequency spectrum of operational taxonomic units (OTU) of microbial communities obtained by the molecular genetic method. Initially, the initial OTU spectrum is converted into an abbreviated spectrum of OTU groups, in which each group combines OTE that are similar in frequency of occurrence. Next, a two-dimensional fractal portrait of the microbial community is constructed, on which each OTU group is represented by a point with coordinates that depend on the frequencies of these groups. A fractal portrait is necessary for conducting a topological analysis of the location of points on it and isolating fractal structures in the microbial community. As a fractal measure, the analysis uses triangles with vertices at the points of the OTU groups. At the same time, the smallest heights of the triangles are selected with the scale of the fractal measure. The construction of triangles and their arrangement in increasing order of scale of the fractal measure allows us to select the first triangles in a row, since they are triangles degenerate into a segment by a straight line. OTU groups included in degenerate triangles are the sought-after determined OTU groups belonging to fractal structures in the microbial community. Their share in the microbial community determines the index of fractal structures (IFS), which also takes into account the uniform distribution of the enzymatic load between OTU.***

In the experiment, the molecular genetic method of NGS was used to study microbial communities in the intestines of broilers of NGS using two types of probiotics.

The obtained frequency-taxonomic spectra were used to calculate the IFS. An analysis of the results showed that probiotics affect the self-organization of microbial communities.

Revised Manuscript Received on November 30, 2019.
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Keywords: ***frequency-taxonomic spectrum, operatively taxonomic units, fractal portrait, microbial community.***

I. INTRODUCTION

Microbial communities of the intestines of birds perform an important trophic function - the extraction of essential nutrients from plant substrates. A long adaptation to the natural conditions of the existence of birds led to self-organization into fractal structures, in which it is possible to carry out complex biochemical transformations of plant substrates with minimal energy costs (Vorobyov, 2011). In the composition of biosystems, microorganisms receive additional systemic advantages and can both organically counteract pathogenic microorganisms and facilitate the penetration of pathogens into plant and animal cells (Vedenyapina, 2014; Fisinin, 2016). Therefore, the study of the self-organization of microbial communities in the intestines of birds in different conditions of keeping and feeding birds is an urgent task. The macroorganism of birds and the microflora of its intestines is an integral biosystem that is in a state of dynamic equilibrium with a relatively constant taxonomic composition of the microbiome. Intestinal microorganisms are involved in vital processes of extracting plant nutrients from both the birds themselves and the microbial community. A number of factors have a significant impact on the efficiency of the transformative activity of the intestinal microbiota, its taxonomic composition and the level of systemic organization: bird age, feed composition, antibiotics, toxins and the presence of pathogenic microorganisms (Biggs et al., 2007; Chichlowski, McBride, 2007; Stanley et al., 2014). In this regard, the intestinal microflora becomes the main object of study of birds and a key indicator of their physiological state, clearly representing their reactions to changes in nutritional diets of feed and the effects of pathogenic microflora. Modern molecular genetic methods have provided researchers with advanced opportunities for studying the microbiome of the intestine of a bird. A taxonomic analysis of the data obtained by the molecular genetic method made it possible to isolate up to 140 bacterial genera at low levels of cell concentration, of which only 10% were identified by the 16S rRNA gene, and the rest belong to new species or even new genera (Amit-Romach et al. 2004; Apajalahti et al., 2004). In addition to taxonomic analysis, fractal analysis (Mandelbrot, 2002) of the frequency-taxonomic spectra of microbial communities of bird intestines allows us to extract quantitative and qualitative information on fractal structures of self-organizing microbial communities of intestinal microflora.

Fractal structures are found in the form of bodies of bodies and animals (Bogatykh, 2012). In microbial communities, fractal structures can be found in the spectra of operational taxonomic objects (OTUs). For example, the number of microorganisms can be a power series: 0.1: 0.01: 0.001 - this means that they correspond to an elementary fractal pattern (Schroeder, 2001). In polymer forms, molecules, fractal structures are observed in distributed interatomic bonds (Abramson, 1999; Karetin, 2016). These organisms are microorganisms in the intestines of birds. These properties are reflected in the fractal ratios of the flows of enzymes from microorganisms, as well as in the synthesis of microorganisms synthesizing these enzymes (Vorobyov, 2012). It determines the minimization of material and energy costs of microbial communities carrying out the synthesis of enzymes and biochemical transformations of plant substrates in the intestines of birds.

Self-organization of microorganisms is necessary for them for coordinated biochemical transformative actions. The microbial community of people should undergo rearrangement, enzymatic synthesis, and biosystem bonds, which are reflected in the structures of microorganisms in the intestines of birds (Stanley, 2014; Fisinin, 2016). If the microbial community of the intestine of birds gradually adjusts to the optimal configuration of the destructive microbial biosystem, complementary to the substrate. Therefore, the fractal structures of microorganisms can be found in various spectra of operational taxonomic objects (OTUs) of the intestine of birds. The qualitative and quantitative composition of fractal structures allows you to control the level of organization of microbial communities in the intestines of birds and the immunity of birds to diseases.

Currently, 6 types of diet components (feed additives) are used in practice to modulate the composition of poultry intestinal microflora: antibiotics, exogenous enzymes, prebiotics, probiotics, synbiotics, phytobiotics (Yang, Y. et al., 2009). The ban in several countries on the use of feed antibiotics (in the EU since 2006), which were previously the main microflora modulating supplement (Castanon, JIR, 2007), has caused increased interest in the remaining types of these supplements, which are intensively studied all over the world. The interest is also spurred by the growing concern of the world community about the problem of resistance of pathogens to antibiotics (Nhung, N.T. et al., 2017).

The objective of the study is to develop a methodology for calculating the index of fractal structures of microbial communities of the intestines of birds according to their frequency-taxonomic spectra of operational-taxonomic units (OTUs) obtained by the molecular genetic method.

II. MATERIALS AND METHODS

To achieve this goal, a zootechnical and physiological experiment was conducted in vivarium conditions of the FSUE Zagorsk EPH VNITIP with 2 probiotics based on the bacteria Lactobacillus. According to the results of the studies, the contents of the blind processes of the intestine were selected, in which the microbial community of chicken intestines was studied by the molecular genetic method NGS (Bruchanov, 2012). The experiment was conducted on 21–36-day-old Cobb 500 cross broilers with feeding diets recommended by VNITIP (Egorov, 2013) see Table 1.

Table – I: Scheme of the experiment on broilers

Group	Features of broiler feeding
1	Complete feed with nutrition corresponding to the recommendations for cross-country (PC)
2	PC + feed supplement at a dose of 1 kg g / t feed containing 10^7 CFU / g Lactobacillus plantarum
3	PC + feed supplement at a dose of 1 kg g / t feed containing 10^7 CFU / g Lactobacillus fermentum

Methodology for calculating the index of fractal structures. The frequency spectrum (OTU) of the microbial community from the intestines of broilers obtained by the molecular genetic method of NGS.

III. RESULTS AND DISCUSSIONS

At the first step of the methodology, the initial OTU spectrum is converted into an abbreviated spectrum of OTU groups, each of which combines OTU that are close in frequency of occurrence. To combine OTE into groups, the entire range of values of the natural logarithm $ln\left(\frac{p_i}{p_{MAX}}\right)$ is divided into intervals (classes) with a step of 0.1, where p_i, p_{max} are the OTU frequencies with number (i) and with the first number (highest frequency). OTUs that fall into one class are combined into one OTU group, and the frequency of occurrence of this group is equal to the sum of the OTU frequencies corresponding to this group. As a result, the initial OTU spectrum (Table 1), consisting of 110 OTUs, is converted to a spectrum consisting of 21 OTU groups.

At the second step of the methodology, according to the data, a two-dimensional fractal portrait of the microbial community is constructed (Vorobyov, 2013 (a); Vorobyov, 2016; Vorobyov 2016 (a)). In the portrait (Fig. 1), the OTE groups are represented by points with coordinates depending on the natural frequencies of the OTE groups. In this case, fractional parts are plotted along the abscissa, and full values are plotted along the ordinate, where qi is the frequency of occurrence of the OTE group with number (i).

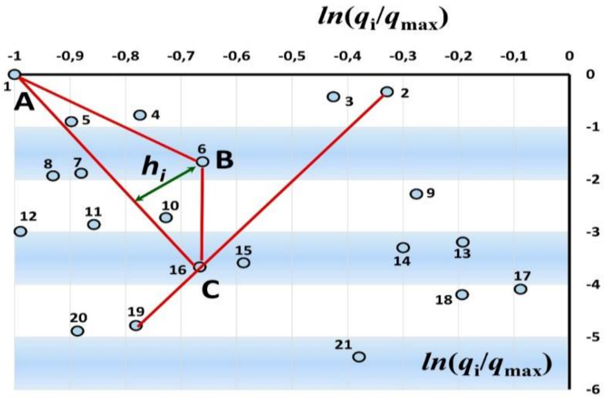

Fig. 1. Fractal portrait of a microbial community.

The points with numbers represent the OTE groups. The triangle Δ ABC is a fractal measure of topological analysis, covering the points of OTE No. 1, 6, 16. The smallest height of the triangle h_i is the scale of the indicated fractal measure. Points No. 2, 16, 19 correspond to a degenerate triangle and an elementary fractal structure.

At the third step of the technique, a fractal topological analysis of the location of points in the portrait is performed (Fig. 1). In the analysis, triangles are used as a fractal measure, at the vertices of which OTE points are located, and the smallest height of such triangles is selected as the scale of the triangular fractal measure. Among all possible constructions of triangles, below we consider only those triangles for which the whole parts of the ordinates $ln\left(\frac{q_i}{q_1}\right)$of the vertices differ in magnitude. For example, the whole parts of the ordinates of points A, B, C differ: 0, -1, -3 (Fig. 1).

As a result of the constructions and calculations in the portrait (Fig. 1), it was possible to isolate M_{TRD} = 10 degenerate triangles (for them hi <0.0065), that is, in this community there are 10 EFS, at the vertices of which there are NGRD = 17 deterministic OTE groups (No. 1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 15, 16, 18, 19, 20, 21). The ratio of the number of determined OTE groups to the total number of OTE groups in the microbial community characterizes the level of participation of microorganisms in biosystem fractal structures and joint biochemical transformations.

$$\frac{C_Y = N_{GRD}}{N_{GRO} = 0{,}810}$$

where C_Y is the coefficient of participation of OTE groups in fractal structures; N_{GRD} = 17 is the number of determined OTU groups present in fractal structures; N_{GRO} = 21 is the total number of OTU groups in the microbial community.

The index of fractal structures of the microbial community (I_F) is calculated as the product of the participation coefficient C_Y (2) and the enzymatic load coefficient C_F (4) (Vorobyov, 2013a; Vorobyev, 2014; Vorobyov, 2016):

$$I_F = \frac{N_{GRD} \cdot M_{min}}{N_{GRO} \cdot M_{TRD}}$$

where NGRD, NGRO - the number of determined OTU groups that formed fractal structures, and the total number of OTU groups in the microbial community; MTRD - the number of determined triangles highlighted in the fractal portrait of the microbial community; Mmin is the minimum number of EFS with a uniform distribution of enzymatic loads between the groups of OTU (2).

According to the data in tables 2 and 3, the index of fractal structures I_F = 0.486 was calculated, that is, in this microbial community, the average level of organization of fractal structures is observed. In cases where it is necessary to compare several microbial communities, in formula (2), the highest N_{GRM} value among those microbial communities that were studied in experiments should be used.

The experience with bird diets showed that the level of self-organization of microbial communities in the intestines of birds depends on the probiotics used. The best indicator of the organization of the microbial community is the experimental version with a probiotic based on Lactobacillus fermentum (Table 2).

Table – 2: Index values of fractal structures of microbial communities in the intestines of chickens

Experience outline	The index of fractal structures of the intestinal microbiota
OP1	0,292
OP1 + Probiotic 1	0,286
OP1 + Probiotic 2	0,354

IV. CONCLUSION

Thus, we can conclude that the developed mathematical model can be used to assess the effectiveness of probiotic feed additives on the microbiota of the intestines of chickens.

ACKNOWLEDGMENT

The study was performed at the FGBOU VO "St. Petersburg State Academy of Veterinary Medicine» with the aid of the Russian Science Foundation Grant (Project No. 18-76-10017).

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Retrieval Number: A5318119119/2019©BEIESP
DOI: 10.35940/ijitee.A5318.119119
Journal Website: www.ijitee.org

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Blue Eyes Intelligence Engineering
& Sciences Publication