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Neural Network Fractal Model to Evaluate the Effectiveness of Antimicrobial Feed Additives in Egg Poultry Farming

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Abstract

The study aimed to develop an artificial neural network fractal model for the quantitative assessment of bioconsolidation indices characterizing the degree of self-organization of the laying hen's intestinal microbiome and to assess the impact of probiotics and prebiotics on such self-organization. At the same time, it is assumed that the consequence of the self-organization of the microbiome of the intestines of birds is the use of their development. To achieve this goal, experiments were carried out on laying hens of the cross "Hisex Brown". At the end of the experiment, the frequency-taxonomic profiles of the microbiota of the caecum of the intestine in laying hens were determined using the high-throughput sequencing method. The fractal principle of self-organization of microbial communities was used to create an artificial computational neural fractal network. Bioconsolidation indices were calculated for each variant of the experiment. In the course of the studies, it was found that a decrease in the values of the bioconsolidation indices demonstrates greater protection of the bird's body when using organic and mineral prebiotics.

Discipline: Bacteriology.

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1 Introduction

Poultry farming is one of the most important areas of agricultural production, providing the population with high-quality dietary food (eggs and poultry meat). Farm poultry is characterized by

relatively fast development, high reproducibility and productivity. Currently, the most poorly studied factor influencing the prolongation of the laying cycle in chickens is the state of the intestinal microflora. It is assumed that the usefulness of the development of their organisms depends on the state of the intestinal microflora of birds. Therefore, it is important to study the effect of feed additives.

The task of modern veterinary science in the field of poultry farming is not only the development of new means for diagnosing and preventing infectious diseases but also the creation of effective methods for their use in large-scale production, the study of the mechanisms of action, taking into account the reaction of the bird's body. For example, it is known that the effectiveness of vaccinations is largely determined by the immunocompetence of birds, which in turn depends on the antioxidant-prooxidant/redox balance in the body (Surai, 2018). In addition, stress-dependent immunosuppression is the most important factor in reducing poultry health (Shini et al., 2010) and reducing its resistance to various pathogens.

Under stress conditions, the self-organization of the intestinal microflora and the interaction of the commensal and pathological components of the microflora are disturbed, which, together with the imbalance of the redox potential in the intestine, leads to pathological changes, expressed in subclinical and clinical inflammations, found in the form of enteritis. It should be emphasized that the disorganization and dysbacteriosis of the intestinal microbiota are considered a key trigger/link in the development of chronic inflammatory phenomena in the intestine (Amoroso et al., 2020).

Currently, hundreds of feed additives with antimicrobial activity have been created and introduced into production in the world. Antimicrobial additives include probiotics, prebiotics, phytobiotics, acidifiers, a number of feed enzymes and complex additives with broad functionality (Gnezdilova, 2020). There is a problem with choosing a reliable method for processing the entire complex of physiological data, including data on the intestinal microbiota of birds, and assessing the impact of these additives on the full development of the bird's body. The aim of the study was to develop an artificial neural network fractal model for the quantitative assessment of bioconsolidation indices characterizing the degree of self-organization of the laying hen's intestinal microbiome and to assess the impact of probiotics and prebiotics on such self-organization (Kochish, 2020).

2 Method

To achieve this goal, experiments were carried out on laying hens (cross "Hisex Brown"; ages 205-207 days), on the basis of the vivarium of the International Laboratory of Molecular Genetics and Poultry Genomics (Federal State Budgetary Educational Institution of Higher Education "Moscow State Academy of Veterinary Medicine and Biotechnology – MBA named after K.I. Skryabin"). The diet of laying hens was organized in accordance with the recommendations of VNITIP (Fisinin et al, 2000; Fisinin, 2016; Egorov et al., 2019).

The work uses a probiotic based on Bacillus megaterium, an organic prebiotic based on lactulose and a mineral prebiotic based on shungite.

Chyme DNA was isolated for subsequent microbiome assessment using the QIAmp Power Fecal DNA Kit (QIAGEN, Germany) on the QIAcube automated system (QIAGEN, Germany). The quality of the isolated DNA was assessed by electrophoresis in a 2% agarose gel with the addition of ethidium bromide (3–5 µl) in a Mini-Sub Cell GT horizontal chamber, Bio-Rad with TAE buffer (Thermo Fisher Scientific, USA). Bromophenol blue for DNA (Thermo Fisher Scientific, USA) was used as a molecular weight marker in a 1:1 ratio with the sample (5 µl of sample to 5 µl of dye) at 220V, 35 minutes. The gel was viewed on a transilluminator, and the amount of DNA was determined on a Qubit 3.0 fluorimeter. Sequencing was performed on an Ion GeneStudio S5 System (Thermo Fisher Scientific, USA). The total number of reads in the analysis is 4 million at 300-400 bp. The belonging of bacteria to a certain taxonomic group was determined using the Ion Reporter network software https://ionreporter.thermofisher.com/ir/.

At the end of the experiment, the frequency-taxonomic profiles of the microbiota of the caecum of the intestine in laying hens were determined using the high-throughput sequencing method.

3 Result and Discussion

3.1 Description of Calculations in Artificial Neural Fractal Network

In the case of organized biosystemic destruction, the fluxes of enzymes and the number of microorganisms that secrete these enzymes correspond to the principle of proportionality to the target restriction sites, along which the decomposition of organic molecules of plant substrates occurs. At the genetic level, the number and location of heterogeneous restriction sites in organic molecules follow fractal patterns (Karetin, 2016; Cattani, 2013; Abramson, 1999). The fractal principle is a fundamental principle (Bogatykh, 2012; Mandelbrot, 2002; Gelashvili, 2010) and is present in the quantitative ratios of the microbial components of the self-organized intestinal microflora of birds. Under ideal conditions, the ratio of frequencies in the microbial profile of bird intestines should also obey the fractal principle, forming a power series of decreasing numbers (Schroeder, 2001). Based on this, we formulated two fractal-stochastic models of the frequency profile of the intestinal microflora in the form of the following series of numbers (s_i).

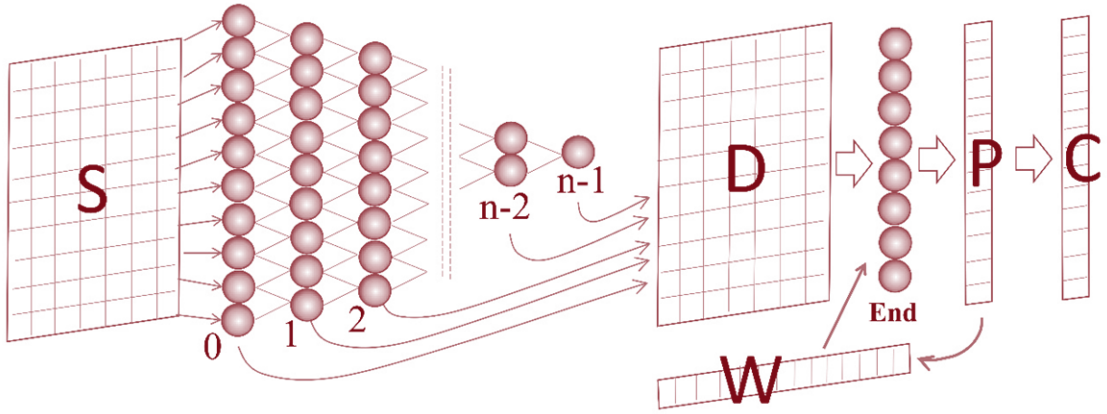
$$s_i \begin{cases} \ell^{-\alpha \cdot i} - fractal\ model \\ \ell^{-\alpha \cdot i^2} - stochastic\ model \end{cases}, \quad (1),$$

where i=1, 2, 3, ... is the serial number of the group of microorganisms in the decreasing frequency profile of the microbial community.

The frequency profile of intestinal microflora groups can be generally characterized by the following indices: Shannon indices (biodiversity indices, IndShen), Simpson indices (dominance indices, IndSimp) (Gorodnichev, 2019; Grishanov, 2010; Chernov, 2015) and fractal (IndBconF) and stochastic (IndBconP) bioconsolidation indices (fractality indices) (Starchenko, 2005; Latypova,

2020; Vorobyov, 2021). IndBconF and IndBconP acquire values in the range 0...1 and depend on the magnitude of the deviation of the envelope of the frequency profile of groups of microorganisms from the model range of numbers (1).

The fractal principle of self-organization of microbial communities was taken into account when creating an artificial computational neural fractal network (Figure1) (Minskiy, 1971; Gafarov, 2018; Sergeev, 2017). The FNS was created for mathematical processing of the frequency profiles of microorganisms and determining the degree of organization of the intestinal microflora using the IndSimp, IndShen, IndBconF, IndBconP indices for various bird diets.



S is a matrix of frequency profiles of microorganisms. D, P – auxiliary matrix and auxiliary vector of numerical data. W is the vector of weight coefficients of the neural layer End. C is the vector of fractal-stochastic indices of bioconsolidation of microbial communities.

Figure 1: An artificial neural fractal network computing self-organization indices of microbial communities

In the structure of neuron No. 0 (Figure 1), there is an increased sorting and ordering in descending order of the initial frequency data ($s_{l.0.i}$) and their logarithmic transformation according to

$$y_{l.0.i} = \begin{cases} \log_2(s_{l.0.i}) - fractal\ model \\ \sqrt{-\log_2(s_{l.0.i})} - stochastic\ model' \end{cases} \quad (2),$$

Where $\sum_{i=1}^{n} s_{l.0.i} = 1$; $s_{l.0.i}$ – frequency of occurrence of a group of microorganisms with the serial number (i) (Table 1); i = 1, 2, ..., n; n is the total number of groups of microorganisms in the biosystem, as well as the number of neurons in layer No. 0; l is the serial number of the experiment variant.

In layers No. 1, ..., n-1, the output values of neurons ($y_{l.k.i}$) are calculated by

$$y_{l.k.i} = y_{l.k-1.i} - y_{l.k-1.i+1} \quad (3),$$

where k = 1, 3, ..., n-1 – numbers of neural layers; n is the number of neurons in the first layer; i = 1, 2, ..., n-k – serial numbers of neurons in the layer with the number (k); l is the number of the experiment variant.

As a result of transformations according to formula (3), we get that in the last layer with the number (n-1) there is only one neuron.

The intermediate matrix D contains the values corresponding to the standard deviations of numbers ($d_{l.k}$) in the layers of neurons.

$$d_{l.k} = \frac{\text{StandardError}(y_{l.k.1}, y_{l.k.2}, \ldots, y_{l.k.n-k})}{2^{k-1}}, \quad (4),$$

where k= 1, 2, ..., n-2 is the number of the neural layer; $d_{l.n}$-1=0; n is the number of groups in the profile of microorganisms, as well as the number of neurons in layer No. 0; l is the serial number of the experiment variant.

At the last stage of NFS calculations, correlation coefficients (r_k) between standard deviations ($d_{l.k}$) and numbers (w_l) of vector W are determined (Figure1). At the first step of calculations, random values are introduced into vector W.

$$r_k = CoefficientCorrelation(d_{1.k}, d_{2.k}, \ldots, d_{l.k}; w_1, w_2, \ldots, w_l), \quad (5).$$

After that, and at the next iteration, the vector P (p_l) is calculated by the formula (6).

$$p_l = \frac{1}{n-2} \cdot \sum_{k=1}^{n-2} g_k \cdot d_{l.k}, \quad (6),$$

where $g_k = \frac{1}{r_D} \cdot (r_k - r_C)$; $r_C = \frac{1}{n-2} \cdot \sum_{k=1}^{n-2} r_k$; $r_D = \sqrt{\sum_{k=1}^{n-2} (r_k - r_C)^2}$; k=1,2, ..., n-2; n – the number of groups in the profile of microorganisms, the number of neurons in layer No. 0.

When training the NFS (Khlivnenko, 2015; Goodfellow, 2018; Nikolenko, 2018), multiple cyclic replacements of the vector W by the vector P and the calculation of the resulting correlation coefficient (R) by formula (7) are used. In this case, at the first step of calculations, random values are introduced into the vector W, and at the next steps, the vector W is replaced by the calculated values of the vector P (see the return arrow from the vector P to the vector W in Figure 1).

$$R = CoefficientCorrelation(w_1, w_2, \ldots, w_{n-2}; p_1, p_2, \ldots, p_{n-2}), \quad (7).$$

The vector C of bioconsolidation indices (c_l) is calculated by formulas (8).

$$c_l = \frac{1}{1 + exp(-a \cdot Abs(p_l))}, \quad (8),$$

where l is the serial number of the experiment variant; a=50 is a constant.

Table 1: Indices of bioconsolidation, Simpson and Shannon indices of the avian intestinal microbial community by experimental variants

Characteristics	Experience Options			
	Control	Organic prebiotic	Probiotic	Mineral prebiotic
Fractal bioconsolidation index, IndBconF	0.39	0.04	0.37	0.17
Stochastic bioconsolidation index, IndBconP	0.46	0.34	0.45	0.43
Simpson Dominance Index, IndSimp	0.63	0.21	0.20	0.20
Shannon Biodiversity Index, IndShen	0.28	0.54	0.55	0.55
Standard error	±0.03	±0.03	±0.03	±0.03

As a result, after ten iterations, the condition R=1 can be reached. At this point, iterations are interrupted, and vector C is considered to be the vector of the desired fractal-stochastic indices of bioconsolidation IndSimp, IndShen, IndBconF, IndBconP of microbial communities in the intestines of birds (Table 1.).

Table 2: Pair correlation coefficients of IndBconF, IndBconP, IndSimp, IndShen indices

	IndBconF	*IndBconP*	*IndSimp*	*IndShen*
IndBconF	1	0.98	0.57	-0.57
IndBconP	0.98	1	0.49	-0.49
IndSimp	0.57	0.49	1	-0.9999
IndShen	-0.57	-0.49	-0.9999	1

4 Conclusion

The fractal bioconsolidation index is smaller in value (IndBconF=0.39; 0.04; 0.37; 0.17, Table 3), the stochastic bioconsolidation index (IndBconP=0.46; 0.34; 0.45; 0.43). This means that the formation of the microbial-organismal biosystem in the intestines of birds is affected to varying degrees by (1) deterministic metagenomic regulatory factors of the bird organism, represented by IndBconF; (2) stochastic regulatory environmental factors presented by IndBconP.

The mineral prebiotic from shungite demonstrates the effect on the IndBconF index (0.39; 0.17; Table 3) in relation to the control, that is, the chain of influence of the mineral shungite on the microbial community of the intestines of birds, which includes the impact on deterministic metagenomic regulatory processes in the body of birds. At the same time, the mineral prebiotic does not affect the impact of external factors that regulate the self-organization of the microbial community in the intestines of laying hens (IndBconP=0.46; 0.43; Table 3).

Correlation of the IndBconF, IndBconP indices with the IndShen index (r=-0.57; -0.49; Table 2) confirms that a decrease in the values of the IndBconF, IndBconP indices means, on the one hand, strengthening the self-organization of the microbial biosystem, and on the other hand, On the other hand, a corresponding increase in the IndShen index, which means the expansion of the biodiversity of the microbial biosystem and the increase in the entropy of the biosystem. Thus, a decrease in the index values demonstrates greater protection of the bird's body when using organic and mineral prebiotics.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

6 Acknowledgement

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