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FRACTAL MODEL OF MICROORGANISMS BIO CONSOLIDATION INTO LAYING HENS INTESTINES BY FEEDING SHUNGITE MINERAL ADDITIVE

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ABSTRACT

This study determines the index of bio consolidation of microorganisms in the intestines of laying hens and assesses the influence of the feed additive from the shungite mineral on the self-organization of the microbial-organismic biosystem in the intestines of birds. Solve this problem was in a vivarium, an experiment on laying hens (cross Hisex Brown). The experimental group's layers differed from the control one by introducing a feed additive from shungite at a dosage of 1 kg per ton of compound feed. To calculate the index of bio consolidation of microorganisms in microbial biosystems and the index of the composition of chemical elements in shungite and mixed feed was built a fractal portrait of the corresponding series of numbers. It is reflecting the sequence of numbers of microorganisms or the content of chemical elements in shungite. It is detected that shungite, with its unique ratio of chemical elemental composition, favorably affects the self-organization of microbial-organismic biosystems in the intestines of laying hens.

Disciplinary: Animal Science, Animal Feed Bioscience and Biotechnology, Veterinary, Biology, Biochemistry.

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1. INTRODUCTION

The chickens' microorganism and microflora of its intestines are an integral microbial-organismic biosystem. It is in a state of dynamic equilibrium with a relatively constant taxonomic composition of the microbiome. Some factors significantly influence the efficiency of the intestinal microbiota's transforming activity, its taxonomic design, and the level of systemic organization: age of chickens, feed composition, antibiotics, toxins, and the presence of pathogenic microorganisms (Biggs et al., 2007; Chichlowski et al., 2007; Stanley et al., 2014). In this regard, the

intestinal microflora becomes the main object of the study of chickens' health status and a key indicator of their physiological state.

Modern molecular genetic techniques have provided researchers with expanded opportunities for studying the gut microbiome of chickens. 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 the 16S rRNA gene identified only 10%, 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 in chicken intestines makes it possible to extract quantitative and qualitative information self-organization microbial-organismic biosystems in the intestines of birds.

In the gastrointestinal tract of chickens, microorganisms and enzymes of compound feed destroy vegetable fiber to monomers. It is most likely that microorganisms achieve high-speed characteristics to decompose plant cellulose only with joint concerted actions. Each enzyme is synthesized in the required amount by only one microorganism (Tsarkova et al., 2016).

In this case, all microorganisms can start simultaneously and release the entire complex of enzymes. With biosystemic microbiological destruction, favorable conditions are to implement the best scenario for the orderly release of enzymes by microorganisms for the economic and rapid decomposition of fiber (Nikonov et al., 2018; Lenkova et al., 2019).

In the case of biosystem destruction, the fluxes of enzymes and the number of microorganisms secreting these enzymes should be proportional in number not only to each other but also to the number of target restriction sites in organic molecules of the decomposed plant substrate. It can assume that if the first, second, and third types of restriction sites are present in organic molecules in a ratio of 3:2:1, then the fluxes of the corresponding enzymes and the number of related microorganisms should also correlate as 3:2:1.

At the genetic level, the location of different types of restriction sites and their relative amounts in organic molecules obey fractal laws (Cattani and Pierro 2013; Karetin, 2016). Based on this, we formulated a unified fractal mathematical model to describe the ratio of microorganisms in destructive biosystems and to describe the content of chemical elements in a mineral feed additive and compound feed, especially in the plant part.

Until the end, an unsolved problem remains to establish the relationship between self-organization microbial-organismic biosystem in birds' intestines and sanitary conditions of keeping and the chemical composition of the food rations of birds. Besides, it is necessary to find a quantitative indicator of the mismatch of biosystem biochemical processes in birds' intestines under biotic and abiotic external influences.

The study determines the index of bio consolidation of microorganisms in the intestines of laying hens and assesses the level of influence of the feed additive from the shungite mineral (Vaisberg et al., 2018) the self-organization of the microbial-organismic biosystem in the intestines of birds.

2. MATERIALS AND METHODS

The experiment was undertaken on laying hens (cross "Hisex Brown") in the vivarium of the International Laboratory of Molecular Genetics and Poultry Genomics of "Moscow State Academy of Veterinary Medicine and Biotechnology - MBA named after K. I. Skryabin". For the experiment, by the principle of analog, we selected the laying hens aged 205-207 days.

The experimental group's layers differed from the control one by introducing a feed additive from shungite at a dosage of 1 kg per ton of compound feed. Shungite has useful sorption property of non-polar mycotoxins and the property of an acidifier. The diet of layers of the parent flock was by the recommendations of All-Russian Research and Technological Poultry Institute.

The fodder additive based on the shungite mineral obtained from the Zazhoginsky deposit in the Republic of Karelia had the chemical composition for crucial micro- and macroelements, see Table 1.

Table 1: The element's content in shungite of Zazhoginskoye mining.

Chemical elements	mg/100 g shungite	mMol/100 g of shungite
C	34.8	2.90
Si	23.07	0.821
Al	6	0.222
Fe	3.5	0.0627
Mg	1.8	0.0741
Ca	1.2	0.0299
S	1.2	0.0374
K	1.01	0.0258
Na	0.25	0.0109
P	0.08	0.00258
Mn	0.022	0.000400
Zn	0.008	0.000122
Cu	0.0058	0.000091

The cecum microbiota's frequency-taxonomic composition in laying hens was determined using the high-throughput sequencing (also called Next-generation sequencing (NGS)) method based on the feeding experience results.

For assessing the influence of external factors on the microbial-organismic biosystem was developed a unified fractal model (from now on referred to as the Fr1 model) (Vorobyov et al., 2019), which is a finite series of numbers decreasing according to a power law:

$$Fr1: 2^b, 2^b \cdot q^{-1}, 2^b \cdot q^{-2}, 2^b \cdot q^{-3}, \dots, 2^b \cdot q^{-N} \quad (1),$$

where $q = 2^{(1-\frac{1}{N})} \dots 2^{(1+\frac{1}{N})}$, $b = -1 \dots 0$, $N = 3 \dots \infty$.

In logarithmic form, the Fr1 model can represent by an arithmetic series of numbers (from now on the Fr2 model):

$$Fr2: b, -\log_2(q) + b, -2 \cdot \log_2(q) + b, -3 \cdot \log_2(q) + b, \dots, -N \cdot \log_2(q) + b \quad (2),$$

where $\log_2(q) = 1 - \frac{1}{N \dots 1 + \frac{1}{N}}$, $b = -1 \dots 0$; $N = 3 \dots \infty$

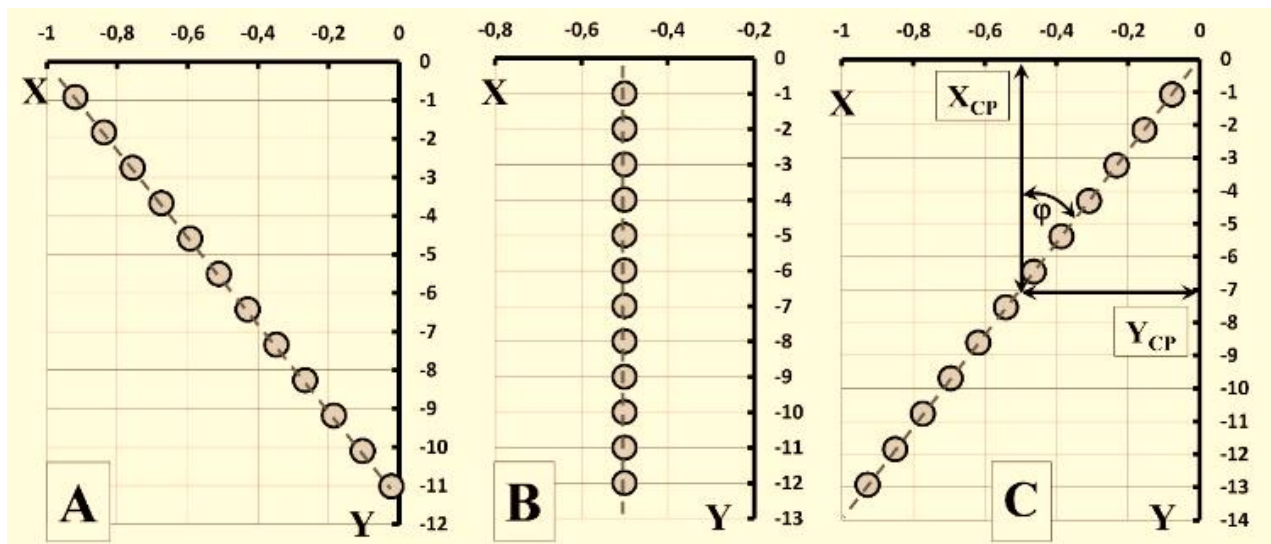
Each numerical position of the Fr1 and Fr2 models specifies not the absolute value of microorganisms in the microbial-organismic biosystem of birds' intestines but their relative numerical status concerning the rest of the microbial components biosystems. It had emphasizes that for any total number of microorganisms in birds' intestines, their percentages remain unchanged.

Unified fractal models Fr1 and Fr2 can apply to numerical series formed from data on the content of chemical elements in Shungite or plants. Each position of the corresponding model series of numbers sets the relative range of chemical elements in Shungite or plants following the fractal power law. The actual numerical series of microorganisms and trace elements will differ from the Fr1 and Fr2 models. Namely, the degree of discrepancy between the model and existing series will further assess the strength of external factors' impact on chickens' microbial-organismic biosystem.

In the fractal portrait, the Fr2 model can be represented by circles, which, regardless of the Fr2 model's parameters, are always located along a straight line (Figure 1). In fractal portraits, the Y- and X-coordinates of the circles depicting the members of the Fr2 model series are

$$\begin{cases} Y_j = -j \cdot \log_2(q) + b \\ X_j = \text{"fractional part"}[-j \cdot \log_2(q) + b] \end{cases} \quad (3)$$

where $q = 2^{(1-\frac{1}{N})} \dots 2^{(1+\frac{1}{N})}$, $b = -1 \dots 0$; $N = 3 \dots \infty$; $j = 2, \dots, N$. The first position ($j = 1$) is not displayed in fractal portraits and is ignored in further calculations, since its coordinates are fixed ($Y = 0, X = 0$).



A - Fr2: $q = 1.89$, $b = 0$, $N = 12$; Fr3: $Y_{CP} = -6.0$, $X_{CP} = -0.47$, $a_m = -0.089$.

B - Fr2: $q = 2$, $b = -0.5$, $N = 12$; Fr3: $Y_{CP} = -6.5$, $X_{CP} = -0.5$, $a_m = 0$.

C - Fr2: $q = 2.11$, $b = 0$, $N = 12$; Fr3: $Y_{CP} = -7.0$, $X_{CP} = -0.50$, $a_m = 0.072$.

The circles represent the portrait of the Fr2 model, and the dashed line represents the Fr3 model.

Figure 1: Fractal portraits of the Fr2 and Fr3 models with different numerical parameters.

Regardless of the values of the Fr2 model's parameters, the circles displaying by their position on the fractal portrait the members of its series are always on straight lines differing only in the inclination (tangent of the angle φ , Figure 1C) to the coordinate axes. This fundamental property of the Fr2 model allows one to approximate the corresponding circles' locations in the fractal portrait by the following linear dependence (from now on, the Fr3 model) (4).

$$\text{Fr3: } X(Y) = a_m \cdot (Y - Y_{CP}) + X_{CP} \quad (4)$$

Where $X_{CP} = \frac{1}{N-1} \cdot \sum_{j=2}^N X_j$; $Y_{CP} = \frac{1}{N-1} \cdot \sum_{j=2}^N Y_j$; X_j and Y_j - coordinates of circles calculated by Formula (3); $j = 2, \dots, N$; a_m - coefficient of linear approximation of circles' location in the fractal portrait. The first position ($j = 1$) in determining the parameters of the Fr3 model is not taken into account since its coordinates are fixed ($X = 0, Y = 0$) and do not determine the specifics of the location of the circles on the fractal portrait.

3. RESULTS AND DISCUSSION

The calculation for the index of bio consolidation of microorganisms in microbial biosystems and the index of the composition of chemical elements in shungite and mixed feed, it is necessary to construct a fractal portrait of the corresponding series of numbers and analyze the arrangement of

circles in the picture depicting the sequence of numbers of microorganisms or the content of chemical elements in shungite or plants.

To construct a fractal portrait of the community of microorganisms in the intestines of chickens (or the content of chemical elements in shungite and plants), it is necessary to first transform the initial numerical data into a series of decreasing dimensionless numbers, similar to how numerical sequence are in the Fr1 or Fr2 models. Table 3 shows an example of the necessary transformations of the initial amounts of microorganisms in chickens' intestines based on the feeding experience results. These data are used to dimensionless form with calculation X- and Y-coordinates of the circles representing the location of groups of microorganisms in the fractal portrait. The data in the first line of Table 2 are not displayed in the picture since they are unchanged for all illustrations.

Table 2: Fractal portrait of microorganisms into intestines.

Microorganism	p_j/p_{max}	Y_j	X_j
Phylum Bacteroidetes	1	0	0
Genus Clostridiales	0.630666	-0.665	-0.665
Phylum Proteobacteria	0.365847	-1.451	-0.451
Genus Lactobacillales	0.353595	-1.500	-0.500
Gamma Proteobacteria Class	0.236076	-2.083	-0.083
Genus Enterobacteriaceae	0.149816	-2.739	-0.739
Phylum Firmicutes	0.073791	-3.760	-0.760
Genus Selenomonadales	0.009471	-6.722	-0.722
Phylum Tenericutes	0.004514	-7.791	-0.791
Phylum Synergistetes	0.001838	-9.088	-0.088
Genus Bifidobacteriales	0.001480	-9.401	-0.401
Phylum Actinobacteria	0.000267	-11.872	-0.872

The Y_j and X_j are coordinates of the circles depicting their position, calculating a group of microorganisms in the fractal portrait, as

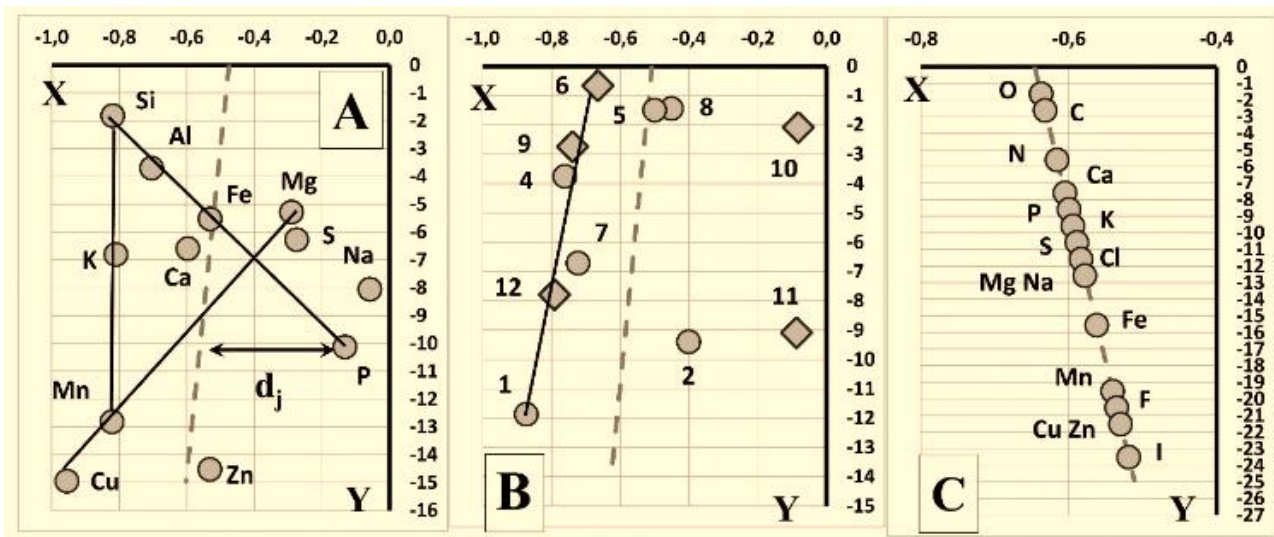
$$\begin{cases} Y_j = \log_2 \left(\frac{p_j}{p_{max}} \right) \\ X_j = \text{"fractional part"} \log_2 \left(\frac{p_j}{p_{max}} \right) \end{cases} \quad (5),$$

where $p_j/p_{max} = 35.37\%$ is the averaged over replicates relative amount of microorganisms with a serial number (j) in a series of numbers and the relative amount of a significant group of microorganisms (in the variant of the experiment with the use of shungite, this is Phylum Bacteroidetes) (Table 3);

$j = 2, \dots, N$; N is the number of controlled groups of microorganisms or analyzed chemical elements.

In fractal portraits, those chemical elements or groups of microorganisms ($j = 1$), which are characterized by the maximum number of p_{max} , are not depicted as circles since their coordinates in the portrait are fixed ($Y_1 = 0, X_1 = 0$) and do not determine specific differences in the structures of the pictures.

Fractal portrait construction (Figure 2B) used Table 3 data for groups of microorganisms in the experiment's variant with shungite. Similarly, a fractal picture (Figure 2A) shows the fractal topology of chemical elements in Shungite. A fractal portrait (Figure 2C) shows the fractal topology of chemical elements plants. In fractal pictures, there are no circles associated with chemical elements or microorganisms characterized by the maximum amount since their coordinates are fixed ($Y = 0, X = 0$) and do not determine specific differences in the structure of the portraits.



Rhombuses represent conditionally pathogenic microorganisms, and circles - useful microorganisms. The dotted lines represent the Fr3 model. Solid lines indicate chemical elements and microorganisms, the ratio of which corresponds to the Fr3 model. d_j is the partial deviation from the fractal model Fr3 of the amount of a chemical element or the number of microorganisms with a serial number (j). 1-Phylum Actinobacteria; 2-Genus Bifidobacteriales; 3-Phylum Bacteroidetes; 4-Phylum Firmicutes; 5-Genus Lactobacillales; 6-Genus Clostridiales; 7-Genus Selenomonadales; 8-Phylum Proteobacteria; 9-Genus Enterobacteriaceae; 10-Class Gamma Proteobacteria; 11-Phylum Synergistetes; 12-Phylum Tenericutes.

A - the content of chemical elements in shungite (Table 2),

Fr3: $X_{CP} = -0.54$, $Y_{CP} = -8.04$, $a_m = 0.009 \pm 0.003$; $I_F = 0.50 \pm 0.03$.

B - the ratio of microorganisms in the experiment with shungite (Table 3),

Fr3: $X_{CP} = -0.55$, $Y_{CP} = -5.19$, $a_m = 0.012 \pm 0.003$; $I_F = 0.55 \pm 0.03$.

C - the ratio of chemical elements in plants (according to literature data),

Fr3: $X_{CP} = -0.58$, $Y_{CP} = -12.8$, $a_m = -0.005 \pm 0.003$; $I_F = 0.88 \pm 0.03$.

Figure 2: Fractal portraits

Plants during their development and the microbial community in the intestines of birds are under the constant influence of biotic and abiotic factors, which leads to destabilization of biosystem processes and deviation of the ratio of microorganisms or the rate of chemical elements in Shungite or plants from the Fr3 model. Therefore, the standard deviation of circles (or rhombuses) from the Fr3 model will then calculate the bio consolidation index of the microbial component of the microbial-organismic biosystem of the avian intestine. The standard deviation of the arrangement of circles associated with the content of chemical elements in shungite will calculate the index of the fractal composition of chemical elements in shungite or plants. Simultaneously, we assume that the index of bio consolidation of microorganisms and the index of the chemical elements in plants and shungite equally reflect the microbial-organismic biosystem's response to external biotic, abiotic influences. In other words, the greater the deviation from the fractal power-law dependence is in the studied numerical series, the greater the disorganization of biochemical processes is in the biosystems under consideration. Thus, bio consolidation and composition indices had calculated using

$$I_F = \left(1 - \sqrt{\frac{1}{N-1} \cdot \sum_{j=2}^{j=N} d_j^2} \right) \cdot R \quad (6)$$

Where $d_j = X_j - a(Y_j - Y_{C.P.})X_{C.P.m}$ (Figure 1A), $X_{CP} = \frac{1}{N} \times \sum_{j=1}^{j=N} X_j$; $Y_{CP} = \frac{1}{N} \cdot \sum_{j=1}^{j=N} Y_j$; $Y_j = \log_2 \left(\frac{p_j}{p_{max}} \right)$, $X_j = \text{"fractional part"} \log_2 \left(\frac{p_j}{p_{max}} \right)$; p_{max} - the relative amount of a group of microorganisms or the relative content of a chemical element with a serial number (j) and the maximum amount of microorganisms or a chemical component of the considered number of digits; a_{min} is the coefficient of linear approximation of the fractal model Fr3. N is the total number of groups of microorganisms or chemical elements.

$R = \frac{N_M}{N}$ - coefficient correcting the deviation of the actual series from the arithmetic series of the Fr2 model. N_M is the minimum number of positions in the series of numbers of the Fr2 model that fully reflect the real ratio of functional groups of microorganisms or the essential chemical elements in the substances under study.

The bio consolidation index values of microorganisms and the index of chemical elements in shungite and plants were calculated using the formula (6) and given in the captions to Figure 2.

4. CONCLUSION

The calculations showed that the index of bio-consolidation of microorganisms in birds' intestines with the addition of shungite to feed ($I_F = 0.55 \pm 0.03$) and the composition of chemical elements in shungite ($I_F = 0.50 \pm 0.03$) are close in value. The coefficients of the Fr3 model for microorganisms ($a_m = 0.012 \pm 0.003$) and for chemical elements in shungite ($a_m = 0.009 \pm 0.003$) and plants ($a_m = 0.005 \pm 0.003$) are also close in value. While in plants, the fractal composition index of chemical elements ($I_F = 0.88 \pm 0.03$) differs significantly from the above values.

Based on the results obtained, shungite, with its unique ratio of chemical elemental composition, favorably affects the self-organization of microbial-organismic biosystems of the intestines of laying hens. The rate of chemical elements in shungite imposes a specific matrix on the ratio of various functional groups of microorganisms in birds' intestines, ensuring the best joint activity of microorganisms in converting organic substrates into nutrients.

The composition index of chemical elements in plants is close to unity. It means that the average statistical content of chemical elements in plants given in the literature reflects plant formation's fundamental processes, determined by fractal power laws. Since shungite is of organic origin, the distribution of chemical elements in shungite also corresponds to fractal laws proven by the obtained data. Taking into account the observed effect of using shungite to bird feed is possible to formulate new criteria for the formulation of chicken feed. They ensure the self-organization of microbial-organismic biosystems in poultry's intestines, rapidly processing vegetable fiber into the necessary nutrients.

5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

6. ACKNOWLEDGEMENT

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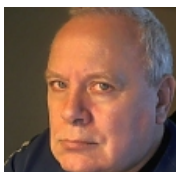
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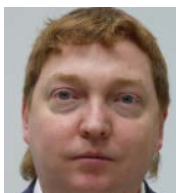
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