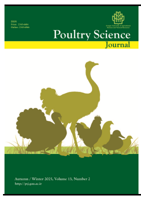

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Effects of a Complex Feed Additive on Productivity and Blood Parameters of Laying Hens Using Stochastic Fractal-based Neural Network Model

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Abstract

Neural networks (NNs) benefit biomedicine and agriculture, especially when relying on the specificity and implementation of stochastic fractal-supported models. In the poultry industry, a particular challenge is the search for an ideal sorbent-based complex additive to minimize the loss of valuable feed components that can be tailored to groups of gastrointestinal microorganisms. The aim of this study was thus to develop and apply a mathematical model and Gaussian NN to analyze productivity and blood parameters of laying hens when administering a complex feed additive from the mineral shungite sorbent, plus a nutritive supplement of brown seaweed meal. We developed and built a computational NN that modelled the stochastic ManyToOne relationship of an array of hens' main blood parameters and performance traits. The results presented herein were that the artificial computational stochastic fractal-based NN (EuclidNN) first effectively analyzed the profiles of operational taxonomic units (OTUs) of the physiological/biochemical blood parameters. Also, correlation coefficients were highly positive in relation to certain zootechnical indicators, suggesting that feed additive intake may have led to changes in these performance traits. Calculations suggested that when implementing the feed additive, the values of the Cognitive Salience Index (*CSI*) vector *vCSI2* declined. Hereby, this vector correlates with, and affects the egg production trait. Moreover, there was a certain relationship between the feed additive intake and feed and water consumption. Further, EuclidNN computed the respective bioconsolidation indices of hens and, simultaneously, processed several profiles of OTUs for all experimental variants. It also contributed to the calculation of bioconsolidation index values for each variant, i.e., a quantitative assessment of the physiological/biochemical blood descriptors, depending on diet. Collectively, the poultry productivity prediction based on the developed model and NN is pivotal as an initial step for future improvements of economically important traits in chickens when using novel and efficient complex feed additives.

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Introduction

For agricultural, and particularly poultry data analysis, neural networks (NNs) present promising and potentially applicable tools for the analysis and synthesis of numerous biological systems (Pitelinskiy and Shimansky, 2013; Jahanmiri and Parker, 2022). One of the major problems of concern is that industrial conditions of livestock exploitation create significant functional stress for the bird's body (Kochish *et al.*, 2019, 2021). Adaptive responses to external stimuli are often stressed, weakening natural defenses, and adversely affecting the health and growth rate of poultry (Young *et al.*, 2022; Kirikovich *et al.*, 2012). One of the ways to alleviate this is through using various feed additives, e.g., mineral-containing substances to stimulate metabolic barriers in the body (Yarovan, 2005; Ghahri *et al.*, 2010; Sharapova, 2011). Contemporary activity in the search for ideal sorbents (i.e., insoluble materials or mixtures thereof that are used to recover liquids through absorption and/or adsorption) aims to reduce the negative effects caused by mycotoxins from the feedstuffs. Minimal loss of useful feed components and adaptability to microorganisms living in various parts of the gastrointestinal tract are also key objectives (Ghahri *et al.*, 2010; Okolelova and Mansurov, 2013; Zasorin *et al.*, 2019; Kochish *et al.*, 2020b; Tyurina, 2022). One of the promising sorbent materials is the mineral shungite (Kochish *et al.*, 2020a; Buryakov *et al.*, 2023), which has a specific set of physical properties and structural characteristics; it is composed of non-crystalline carbon with a metastable structure, being incapable of graphitization (Ignatov and Mosin, 2013; Tukhbatov, 2013). Shungite rock has good adsorption properties due to its developed and active surface and is capable of acting as a catalyst or catalyst carrier in organic synthesis reactions (Gorodnichev *et al.*, 2019; Vats *et al.*, 2016; Zasorin *et al.*, 2019). Recently, attention has been also drawn to marine brown algae as a nutrient and efficient feed additive. These algae are rich in protein, vitamins and other biologically active substances that play an important role in the body's metabolism (Sharapova, 2011; Buryakov *et al.*, 2023; for review see Kochish *et al.*, 2021).

To model complex stochastic biochemical processes occurring in a bird's body, a computational NN can be employed that includes procedures of multivariate mathematical statistics, i.e., correlation, cluster and discriminant analyses (Meireles *et al.*, 2003; Colbrook *et al.*, 2022; Taye, 2023). One of the instrumental solutions for constructing appropriate mathematical models to describe certain processes, including biologically significant ones, can be stochastic fractal-based mathematical models. These comprise hybrid mixing methods for the mathematical modeling (Moroz and Maslovskaya, 2020; Zaikina *et al.*, 2022) applicable to various (e.g., biological) processes. Previously, we developed a concept of fractal conformity-based bioconsolidation index, which varies within 0 … 1 depending on the efficiency of coherent biological processes in the body, and tested an artificial NN modelling approach (Kochish *et al.*, 2020b, 2023; Pukhalskiy *et al.*, 2023; Vorobyov *et al.*, 2023a,b), suggesting reasonable prospects of their applications in biological studies. Computational NNs are designed to model a ManyToOne relationship that, for instance, connects a multiple set of heterogeneous bird blood parameters of different dimensions with one single dimensionless numerical indicator for the cognitive significance of the data being studied, i.e., the Cognitive Salience Index (CSI = 0 ... 10; Sutrop, 2001; Mascarenhas, 2018). In one particular case, the dimensionless CSI index can be interpreted as an indicator of an increase/decrease in the intensity of biochemical processes in the bird's body that determine the egg performance of laying hens. Furthermore, an important stage in the implementation of an NN in poultry-related research is setting up/training the NN and checking/validating it based on a correlation analysis of the CSI values with egg production indicators of layers. After successful NN training and validation, it is feasible to estimate the feed additive intake effects (positive or negative) on the egg performance by increasing/decreasing the CSI values.

Considering the remarkable adsorption properties of shungite rock and the nutritional benefits of incorporating brown algae into poultry diets, a combination of these supplements can be most attractive for practical use (Sharapova, 2011; Buryakov *et al.*, 2023). Here, we thus applied an NN and hybrid stochastic fractal-based approach to the mathematical process of analyzing the poultry productivity and blood parameters under the influence of a complex of these two feed supplements. The goal of the present study was therefore to develop the appropriate mathematical model and NN based on this to analyze the performance and blood parameters of laying hens in response to the intake of a complex additive that combined shungite and brown algae, estimating the respective CSI and bioconsolidation indices.

Materials and Methods

Experimental birds, performance traits and blood parameters

This study was conducted at a large poultry farm located in Leningrad Oblast, Russia, using Hisex White commercial cross layers (Laptev *et al.*, 2017) at the starting age of 49 weeks. Birds were kept in group cages and fed the basic diet containing grain, root crops, green grass, and a vitamin-mineral feed premix. According to the existing standards, there were 5 laying hens in each cage. The cage had a standard size, with the dimensions of the total seating area being 490 × 580 × 550 mm. This allowed for a regulated density of hens in the cage, which meets the general requirements that are very strictly observed at this poultry farm. The control (C) group totalled 225,378 hens. The experimental (E) group numbered 218,125 birds fed the basic diet supplemented with the following complex feed additive: 0.7 kg shungite and 0.3 kg brown algae (as dried *Fucus vesiculosus* seaweed meal) per 1-ton fodder. The objective of this feeding experiment was to assess the effect of the combination of these two supplements, i.e. their synergistic impact, considering that the two feed ingredients, different in their essence, are expected to have non-overlapping effects from their use. In particular, the mineral shungite is an inorganic sorbent substance that is not digested in the hen's body, while algae, of an organic (plant) nature, when assimilated in the chicken's body, go through digestive processes in the same way as other plant (and animal) components of the compound feed. The chosen optimal dosages and the ratio of the two supplements were based on our and other preliminary tests (Sharapova, 2011; Buryakov *et al.*, 2023) and taking into account an overall economy of the compound feed recipe that poultry enterprises in Russia adhered to for keeping feed costs reasonable. For the NN analysis, the six most important zootechnical indicators of egg production, feeding and watering of laying hens were also taken as follows: egg production rate (in %), egg weight (in g), proportions of cracked, leaking and dirty eggs (in %), feed conversion (in kg per 10 eggs produced), feed consumption (in g per hen), and water consumption (in mL per hen), plus their four derivative indices (ratios).

We studied the effect of the complex additive on older laying hens in their third feeding phase (i.e., at 75 weeks of age and after), which is considered critical and corresponds to a physiological state of decline in egg production rate and, accordingly, overall productivity. Using 0.5 to 1 mL of blood per sample, three 75-week-old females were sampled from the control and experimental groups (designated C1 and E1, respectively). Five hens per group were then sampled to analyze 12 blood parameters (Table 1) in the middle (86 weeks of age; designated C2 and E2, respectively) and at the end of the feed additive administration study (94 weeks of age; designated C3 and E3, respectively). Accordingly, twelve physiological/biochemical blood characteristics of the laying hens were assessed (Table 1), including the following most informative indices:

- Descriptors of erythrocytes, leukocytes and platelets using Goryaev's grid-equipped counting chamber (Tietz, 1997);
- Blood leukogram using microscopy, May-Grünwald fixative and Romanowsky azure-eosin dye to stain blood smears (Tietz, 1997);
- Serum hemoglobin level using the hemoglobin cyanide method, and the formula-assisted cell-color ratio (Tietz, 1997);
- Phagocytosis indices using a microscopic method and *Staphylococcus aureus* strain 209 culture inactivated by heat and standardized via an optical turbidity standard (Menshikov, 1997).

Data analysis principles using NN

Super-new artificial intelligence (AI) computer programs (D'Addona, 2014; Gharajeh, 2018; Dall'Alba *et al.*, 2022) were used with the respective computational NNs to search, e.g., for feed additives that ensure minimal loss of useful feed components and modulate the gastrointestinal microflora to ensure a complete symbiosis of microorganisms with the animal's body (e.g., Kochish *et al. et al.*, 2020b, 2023; Pukhalskiy *et al.*, 2023). In this regard, the following methods can be considered most relevant (Pitelinskiy and Shimansky, 2013; Taye, 2023):

- Standard statistical methods based on mathematical instrumental regression analysis that optimize specific experimental parameters by applying an appropriate evaluation function.
- Methods based on the use of functional expansions in a Fourier series (Garrapa, 2018). Herein, it is taken into account that the experiment is a commutative process and can be transformed by a sum of functions that describe its polynomials with a given accuracy (e.g., trigonometric functions).
- Partial modelling and formal grammars (Pitelinskiy and Shimansky, 2013).

A sequence of s elements can be considered as an "approximation" in that identical oscillations are often observed in biological systems, differing only in magnitude and time of occurrence, i.e., scale-invariance (Grosu *et al.*, 2023). To describe this, the concept of diversity as a heterogeneous destructive body is employed. For a complete description of diversity, the entire spectrum of dimensions is necessary since, unlike a common mathematical decomposition, it is not enough to introduce only one value, the fractal index d. These dimensions are usually infinite numbers and other time intervals and frequencies of a sample can be further analyzed. Current trends, i.e., specific signal sequences, can then be examined in more detail using, for instance, frequency analysis (Casals *et al.*, 2005; Pitelinskiy and Shimansky, 2013). Conventional experiments are based on inertial analysis and use flexible linear statistical models. However, NNs are inherently nonlinear and do not require knowledge of the correlation between input data and output data. This makes them more promising than traditional methods (Meireles *et al.*, 2003). Although simulations involve the use of multilayer sensor devices (Rosenblatt, 1962; Minsky and Papert, 1969/1988), the use of closed-loop NNs is not always recommended due to short-term memory and difficulty of use (Pitelinskiy and Shimansky, 2013; Colbrook *et al.*, 2022).

Table 1: Mean values (±*SEM*[1]) of 12 blood parameters in hens by four experiment variants (C2, C3, E2, and E3) and correlation coefficients (*R*) with *vCSI1* and *vCSI2* vectors[2]

Blood parameters	Experiment variants[3]				***R* (*SEM* = ±0.01)**	
	C2	C3	E2	E3	*vCSI1*	*vCSI2*
Erythrocyte count (mln/μL)	2.88±0.19	3.10±0.47	2.60±0.25	3.19±0.36	0.83 (*P*= 0.021)	−0.78 (*P* 0.040)
Erythrocyte sedimentation rate (mm/h)	1.20±0.40	1.20±0.40	1.20±0.40	1.60±0.49	0.67 (*P*= 0.098)	−0.62 (*P* 0.137)
Leukocyte count (thousand/μL)	30.26±4.13	35.85±3.93	31.22±2.78	30.34±8.74	0.44 (*P*= 0.324)	−0.39 (*P*= 0.391)
Platelet count (thousand/μL)	71.84±11.34	65.71±10.84	54.62±16.31	62.67±22.58	−0.07 (*P*= 0.889)	0.12 (*P*= 0.801)
Eosinophils	2.20±1.10	1.20±0.40	1.80±0.75	0.40 ± 0.49	−0.90 (*P*= 0.006)	0.95 (*P*= 0.001)
Pseudoeosinophils	6.60±1.02	14.60±4.92	12.00±1.67*	18.80±2.93	0.93 (*P*= 0.003)	−0.88 (*P*= 0.010)
Monocytes	0.40±0.49	1.00±0.00	0.80±0.75	0.80±0.40	0.74 (*P*= 0.056)	−0.69 (*P*= 0.086)
Basophils	1.00±0.89	0.80±0.40	1.20±0.40	0.80±0.75	−0.78 (*P*= 0.041)	0.83 (*P*= 0.022)
Lymphocytes	89.60±2.33	82.40±4.80	84.20±2.64	79.20±2.79	−0.86 (*P*= 0.014)	0.91 (*P*= 0.005)
Hemoglobin (g/L)	80.00±5.48	89.20±8.70	85.60±4.18	83.00±8.85	0.50 (*P*= 0.249)	−0.45 (*P*= 0.308)
Cell-color ratio	1.68±0.21	1.79±0.44	1.99±0.11	1.59±0.28	−0.38 (*P* = 0.395)	0.44 (*P* = 0.328)
Phagocytic index (a.u.)[4]	10.62±0.87	9.57±1.31	9.93±1.45	11.01±0.92	0.08 (*P* = 0.867)	−0.03 (*P* = 0.955)

[1] *SEM*, standard error of the mean. [2] *vCSI1* and *vCSI2*, vectors containing the values of the *CSI1* and *CSI2* cognitive salience indices for the experiment variants. [3] Experiment variants corresponded to the second and third blood sampling points (at 86 and 94 weeks of age) for the control (C2 and C3) and experimental (E2 and E3) groups of laying hens, respectively. [4] a.u., arbitrary units. * Significant difference between the respective C2 and E2 values (at $P < 0.05$).

General NN and stochastic fractal-based model description

The mathematical research was carried out using NN analysis and stochastic fractal-based modelling (Garrapa, 2018; Moroz and Maslovskaya, 2020; Zaikina *et al.*, 2022). The NN and its elements we report here are subject to protecting the intellectual property of the authors (Vorobyov *et al.*, 2023b). NN data mining implies a machine learning algorithm and data analysis technique that mimics the functionality of the human brain (Khlivnenko, 2015; Goodfellow *et al.*, 2016). This approach involves a large number of interconnected nodes capable of processing and transmitting information. Because of this, NNs can be typically used to process large amounts of information and identify patterns among the analyzed datasets (D'Addona, 2014), the latter being, in our case, the physiological/biochemical blood parameters of laying hens.

When examining blood parameters as operational taxonomic units (OTUs), it was important to determine how the statistics of these datasets differ from the conventional Gaussian statistics (i.e., normal distribution) (Wentzel, 1999). It was assumed that any deviation of the actual data statistics from Gaussian statistics would be indicative of the regulation of physiological/biochemical processes in the blood, e.g., changes in the levels of erythrocytes, leukocytes, platelets, etc., that are controlled by the bird's body. Deviations from the normal distribution were assessed by the magnitude of the highest central moments in the empirical distributions of the measured values (Rosenblatt, 1962; Popov, 2013).

The physiological/biochemical characteristics of the hen's blood can be characterized by Shannon entropy indices (*IndShen*) also considered biodiversity indices (Grishanov and Grishanova, 2010; Chernov *et al.*, 2015; Gorodnichev *et al.*, 2019) and by bioconsolidation indices (*IndBconI*) of the bird's body and its immune system status (Kochish *et al.*, 2020b, 2022, 2023). With increasing entropy (increasing *IndShen* index), the physiological/biochemical characteristics of blood across replicates are levelled out, and their dispersion decreases. Therefore, it can be assumed that the positive correlation of the *IndShen* index with the *IndBconI* index means that the corresponding characteristic of hen's blood may be a signal indicator of the bioconsolidation of the immune status.

To calculate the distribution moments for the empirical blood parameter data used as OTUs and compute the respective bioconsolidation indices of the hen's body and its immune status, an artificial Gaussian NN (GNN) was implemented (Minsky and Papert, 1969/1988; Filntisi *et al.*, 2013; Sergeev and Tarasov, 2017; Mascarenhas, 2018; Gafarov and Galimyanov, 2018) (Figure 1). This was based on a stochastic fractal-based model (Moroz and Maslovskaya, 2020; Zaikina *et al.*, 2022) for defining the flow of physiological/biochemical processes in the bird's body.

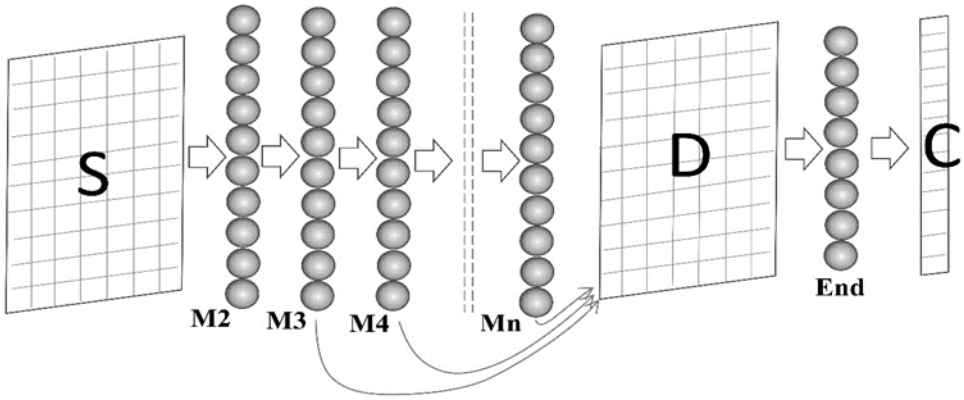

Figure 1. Artificial Gaussian neural network for calculating bioconsolidation indices of the bird's body and its immune system status. S, matrix of physiological/biochemical blood parameters; D, matrix of statistical moments of blood parameters; and C, vector of bioconsolidation indices.

Results

Constructing NN and stochastic fractal-based model

In the course of developing the artificial GNN further called *EuclidNN* (Figure 2), we built a computational NN that modelled the stochastic ManyToOne relationship of an array of hens' main blood parameters and performance traits (used as OTUs) with one unique dimensionless characteristic, *CSI* (Sutrop, 2001; Mascarenhas, 2018). Values of the *CSI1* and *CSI2* indices for the four experiment variants were transformed into *vCSI1* and *vCSI2* vectors (Tables 1 and 2).

The most informative poultry performance indicators and correlation coefficients with vectors *vCSI1* and *vCSI2* are summarized in Table 2.

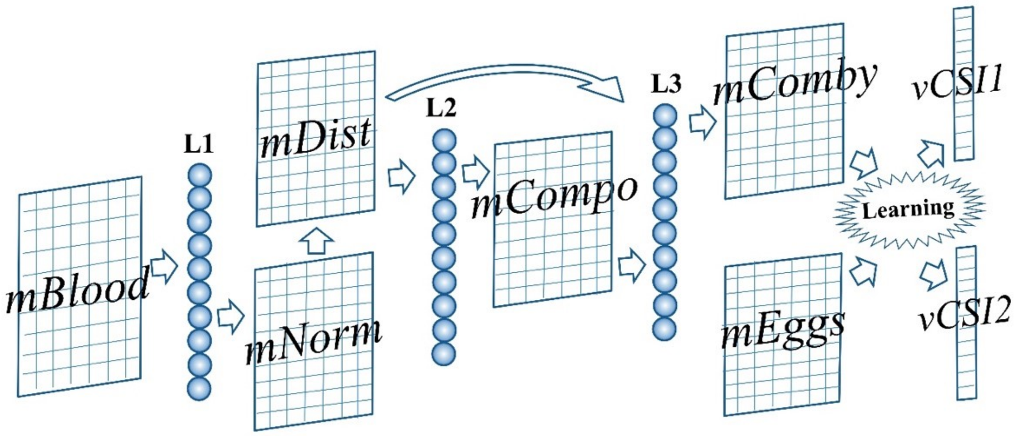

Figure 2. Chart flow of the EuclidNN computational neural network. *mBlood* is a matrix of 12 key physiological/biochemical blood indicators (Table 1). *mEggs* is a matrix of eight physiological performance-related indicators (Table 2). *mNorm* is a matrix of normalized values of the *mBlood* matrix. *mDist* is a matrix of Euclidean distances of four experimental variants based on blood parameter data. *mCompo* is a matrix of orthogonal components in the space of blood parameter data. *mComby* is a matrix containing the results of a nonlinear combinatorial transformation of data from matrices *mDist* and *mCompo*. *vCSI1* and *vCSI2* are vectors containing the values of the *CSI1* and *CSI2* cognitive salience indices for the experiment variants. L1, L2 and L3 are layers of artificial neurons that perform matrix transformations of the initial and intermediate data.

Table 2. Mean values of eight key zootechnical indicators (i.e., hen productivity, feeding and watering) by four experiment variants (C2, C3, E2 and E3) and correlation coefficients (*R*) with vectors *vCSI1* and *vCSI2*

Indicators	Experiment variant[1]				***R* (*SEM* = ±0.01)**	
	C2	**C3**	**E2**	**E3**	***vCSI1***	***vCSI2***
Egg production rate, %	88.7	77.9	85.4	71.8	−0.92 (P = 0.003)	0.98 (P< 0.001)
Egg weight, g	64.3	66.5	64.8	65.5	0.85 (P = 0.016)	−0.80 (P= 0.032)
Cracked eggs, %	4.0	8.8	8.8	12.4	0.83 (P = 0.020)	−0.78 (P= 0.039)
Leaking eggs, %	0.21	0.41	0.33	0.54	0.94 (P = 0.002)	−0.89 (P= 0.008)
Dirty eggs, %	2.9	9.7	4.1	12.6	0.99 (P < 0.001)	−0.94 (P= 0.002)
Feed conversion, kg/10 eggs	1.46	1.64	1.63	1.72	0.84 (P = 0.019)	−0.78 (P= 0.037)
Feed consumption, g/hen	130	128	139	124	−0.66 (P = 0.109)	0.71 (P= 0.074)
Water consumption, mL/hen	250	248	250	228	−0.68 (P = 0.092)	0.73 (P= 0.061)
vCSI1 vector (*SEM* = ±0.2)	2.5	6.7	3.4	7.4	1.00 (P < 0.001)	−0.95 (P= 0.001)
vCSI2 vector (*SEM* = ±0.2)	7.5	3.3	6.6	2.6	−0.95 (P = 0.001)	1.00 (P< 0.001)

[1] Experiment variants corresponded to the second and third blood sampling points (at 86 and 94 weeks of age) for the control (C2 and C3) and experimental (E2 and E3) groups of laying hens. *SEM*, standard error of the mean.

The constructed multilayer EuclidNN included three artificial neuron layers L1, L2 and L3 that executed the initial and intermediate data matrix transformations (Figure 2) as outlined below.

L1 neuron layer

This performed normalization of the *mBlood* matrix data (Figure 2) (Everitt *et al.*, 2011; Mascarenhas, 2018) that contained digital data on the blood parameters of birds (OTUs) in the four experiment variants (Table 1). The results of normalization were stored in the *mNorm* matrix according to the following Equation 1:

$$mNorm_{jk} = \frac{mBlood_{jk} - \frac{1}{4}\cdot\sum_{k=1}^{k=4} mBlood_{jk}}{\sqrt{\sum_{k=1}^{k=4}\left[mBlood_{jk} - \frac{1}{4}\cdot\sum_{k=1}^{k=4} mBlood_{jk}\right]^2}} \quad (1)$$

where $mBlood_{jk}$ are *mBlood* matrix values; j = 1, 2, …, 12 are ordinal numbers of 12 blood parameters (Table 1); and k = 1, 2, …, 4 are ordinal numbers of the four experiment variants that conformed to C2, C3, E2 and E3, respectively (Tables 1 and 2).

The L1 neuron layer also computed the *mDist* matrix, i.e., the matrix of Euclidean distances between the four experiment variants in the 12-dimensional space of birds' blood parameters (Yeung and Ruzzo, 2001; Everitt *et al.*, 2011) using the following Equation 2:

$$mDist_{mn} = \sqrt{\frac{1}{12}\sum_{j=1}^{j=12}\left(mNorm_{jn} - mNorm_{jm}\right)^2}, \quad (2)$$

where m, n = 1, 2, …, 4 are ordinal numbers of the four experiment variants that conformed to C2, C3, E2 and E3, respectively (Tables 1 and 2); and j = 1, 2, …, 12 are ordinal numbers of 12 blood parameters (Table 1).

L2 neuron layer

This computed the *mCompo* matrix, i.e., the matrix of principal components (orthogonal eigenvectors) in the 12-dimensional space of birds' blood indices using the *mDist* matrix data and a standard computational procedure (Yeung and Ruzzo, 2001; Jolliffe, 2002; Schmidhuber, 2015) as follows:

$$mCompo = EigenVectors(mDist). \quad (3)$$

L3 neuron layer. This computed the *mComby* matrix containing numerical data that were obtained by different variants of the nonlinear combination of the *mDist* and *mCompo* matrix data. The *mComby* matrix data were intended for training tuning of the EuclidNN model and for calculating the desired *vCSI1* and *vCSI2* index vectors using hens' blood parameters.

When training the EuclidNN neural network (Nikolić *et al.*, 2012; Schmidhuber 2015; Widrow *et al.*, 2013; Nikolenko *et al.*, 2018), the second orthogonal component (*mCompo2*, i.e., the second row of the *mCompo* matrix) in the 12-dimensional space of blood indices was chosen because the projection of blood indices onto the *mCompo2* component maximally correlated with performance indices (Table 2) (Gao *et al.*, 2012; Batushansky *et al.*, 2016). Using the values of the *mCompo2* component, the values of the vectors *vCSI1* and *vCSI2* (Tables 1 and 2) were calculated using the following Equations 4 and 5:

$$vCSI1_k = mCompo2_k \cdot 4.17 + 5, \quad (4)$$
$$vCSI2_k = -mCompo2_k \cdot 4.17 + 5, \quad (5)$$

where k = 1, 2, …, 4 are ordinal numbers of the four experiment variants that conformed to C2, C3, E2 and E3, respectively (Tables 1 and 2).

In addition, the correlation coefficients (R) of *vCSI1* and *vCSI2* vectors with blood parameters and performance indicators were calculated as summarized in Tables 1 and 2.

To compute the statistical moments of blood indices in the layers of neurons M2, M3, M4, ..., Mn, the moments ($d_{l.j}$) forming the matrix of moments D (Figure 1) were calculated using the following Equations 6 and 7:

$$d_{l.j} = \left[\frac{1}{N-1} \cdot \sum_{k=1}^{k=N} Abs\left(s_{l,k} - m_l\right)^j \right]^{1/j} \Big/ m_l \quad (6),$$

$$m_l = \frac{1}{N} \sum_{k=1}^{k=N} s_{l.k} \quad (7),$$

where m_l ($s_{l.k}$) are physiological/biochemical blood parameters; k = 1, 2, …, N are numbers of replicates of blood parameters in samples; j = 2, 3, …, N–2 are numbers of statistical moments of blood parameters; N is the number of blood parameter values in a sample; and l is the serial number of the four experiment variants.

Subsequently, *IndBconI* (c_l) indices were calculated in the End neuron layer (Figure 1) using the following Equation 8:

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

where the respective additional indices were computed as follows:

$$p_l = \sum_{j=1}^{j=N-2} \left(\frac{d_{j.l} \cdot g_{j.l}}{IndShen_l} \right), \quad g_j = \frac{1}{r_D} \cdot \left(\sum_{l=1}^{l=M} d_{l.j} - r_C \right),$$

$$r_C = \frac{1}{N} \sum_{j=2}^{j=N-2} \left(\sum_{l=1}^{l=M} d_{l.j} \right), \quad r_D = \sqrt{\sum_{j=2}^{j=N-2} \left(\sum_{l=1}^{l=M} d_{l.j} - r_C \right)^2},$$

$$IndShen_l = \frac{1}{\log(N)} \sum_{k=1}^{k=N} \left[\frac{s_{l.k}}{m_l} \cdot \log\left(\frac{s_{l.k}}{m_l} \right) \right],$$

$$m_l = \frac{1}{N} \sum_{k=1}^{k=N} s_{l.k};$$

j = 2, 3, …, N−2 are numbers of statistical moments of physiological/biochemical blood parameters; k = 1, 2, …, N are numbers of blood parameter values in a sample; l is serial number of the four experiment variants; and a is a constant.

EuclidNN computational algorithm implementation to analyze experimental data

The input data for calculations using EuclidNN (Figures 1 and 2) (Rosenblatt, 1962; Meireles *et al.*, 2003; Anastasiadis, 2005; Filntisi *et al.*, 2013; Mascarenhas, 2018; Colbrook *et al.*, 2022; Taye, 2023) were generated in the Excel environment and are presented in Tables 1 and 2).

At the first stage, the appropriate immune bioconsolidation indices were calculated from the most informative physiological/biochemical blood parameter data using the GNN artificial intelligence process and are summarized in Table 3.

Then, the correlation coefficients between the *IndBconI* indices and performance parameters of laying hens were computed as shown in Table 4. As can be seen from Table 4, there were high correlation coefficients (positive or negative) between certain performance indicators and blood parameter bioconsolidation indices. For example, the egg mass yield indicators, i.e., the product of mean egg weight and egg production, positively correlated with the *IndBconI* values for hemoglobin (R = 0.998, 0.838 and 0.916) and platelet counts (R = 0.878, 0.777 and 0.993).

Finally, the values of the vectors *vCSI1* and *vCSI2* were computed using EuclidNN (Tables 1 and 5). In particular, when administering the shungite–seaweed feed additive, the *vCSI1* vector values raised as follows: *vCSI1*(C2) = 2.5 < *vCSI1*(E2) = 3.4; and *vCSI1*(C3) = 6.7 < *vCSI1*(E3) = 7.4 (Table 5). The correlation coefficients (Table 5) for the *vCSI1* vector were highly positive in relation to certain zootechnical indicators (R = 0.83 to 0.99), suggesting that the feed additive intake may have led to changes in these performance indicators.

Table 3: Bioconsolidation indices (*IndBconI*) for five main blood parameters according to the four experiment variants[1]

Blood indicators[2]	C2	C3	E2	E3
Erythrocyte count	0.65	0.77	0.52	0.53
Leukocyte count	0.37	0.30	0.41	0.48
Platelet count	0.27	0.47	0.35	0.47
Hemoglobin	0.53	0.62	0.56	0.71
Cell-color ratio	0.81	0.92	0.50	0.67

[1] Experiment variants corresponded to the second and third blood sampling points (at 86 and 94 weeks of age) for the control (C2 and C3) and experimental (E2 and E3) groups of laying hens. *SEM* = ±0.02, standard error of the mean.

Table 4. Correlation coefficients (*R*) between blood parameter bioconsolidation indices (*IndBconI*) and performance indicators

Performance indicators	*IndBconI* Erythrocyte count	Leukocyte count	Platelet count	Hemoglobin	Cell-color ratio
Egg production rate, %	0.77 ($P = 0.026$)	−0.39 ($P = 0.337$)	0.47 ($P = 0.235$)	0.40 ($P = 0.323$)	0.93 ($P < 0.001$)
Mean egg weight, g	0.87 ($P = 0.005$)	−0.54 ($P = 0.167$)	0.35 ($P = 0.391$)	0.23 ($P = 0.588$)	0.98 ($P < 0.001$)
Mean egg mass yield[1]	0.05 ($P = 0.898$)	0.38 ($P = 0.355$)	0.77 ($P = 0.025$)	0.93 ($P < 0.001$)	0.32 ($P = 0.435$)
Mean egg mass yield to feed consumption ratio	0.72 ($P = 0.045$)	−0.36 ($P = 0.375$)	0.73 ($P = 0.037$)	0.58 ($P = 0.134$)	0.81 ($P = 0.014$)
Mean egg mass yield to water consumption ratio	−0.61 ($P = 0.111$)	0.38 ($P = 0.360$)	−0.77 ($P = 0.026$)	−0.59 ($P = 0.125$)	−0.68 ($P = 0.065$)
Total nonmarket eggs, %	−0.18 ($P = 0.673$)	0.17 ($P = 0.694$)	−0.86 ($P = 0.006$)	−0.58 ($P = 0.129$)	−0.06 ($P = 0.892$)
Feed conversion, kg per 10 eggs	−0.79 ($P = 0.019$)	0.75 ($P = 0.033$)	−0.57 ($P = 0.137$)	−0.19 ($P = 0.660$)	−0.67 ($P = 0.069$)
Feed conversion, kg per 1 kg egg mass yield	−0.68 ($P = 0.065$)	0.48 ($P = 0.228$)	−0.73 ($P = 0.038$)	−0.50 ($P = 0.206$)	−0.71 ($P = 0.050$)
Feed consumption, g per hen	−0.79 ($P = 0.020$)	0.55 ($P = 0.159$)	0.30 ($P = 0.476$)	−0.20 ($P = 0.637$)	−0.92 ($P = 0.001$)
Water consumption, mL per hen	−0.47 ($P = 0.238$)	0.18 ($P = 0.675$)	−0.76 ($P = 0.028$)	−0.70 ($P = 0.053$)	−0.63 ($P = 0.094$)

[1] The product of mean egg weight by egg production characterizes the egg mass yield as a whole. *SEM* = −0.08 … 0, standard error of the mean.

Table 5. Mean values of vectors *vCSI1* and *vCSI2* by four experiment variants (C2, C3, E2 and E3) and correlation coefficients (*R*) with eight key zootechnical indicators (i.e., hen productivity, feeding and watering)

Vectors	Experiment variant[1]				*R* (*SEM* = ±0.01)	
	C2	C3	E2	E3	*vCSI1*	*vCSI2*
vCSI1 (*SEM* = ±0.2)	2.5	6.7	3.4	7.4	1	−1
vCSI2 (*SEM* = ±0.2)	7.5	3.3	6.6	2.6	−1	1

Vectors	*R* values (*SEM* = ±0.01) for indicators							
	Egg production	Egg weight	Cracked eggs	Leaking eggs	Dirty eggs	Feed conversion	Feed consumption	Water consumption
vCSI1	–	0.84	0.83	0.94	0.99	0.83	–	–
vCSI2	0.98	–	–	–	–	–	0.69	0.74

[1] Experiment variants corresponded to the second and third blood sampling points (at 86 and 94 weeks of age) for the control (C2 and C3) and experimental (E2 and E3) groups of laying hens. *SEM*, standard error of the mean.

Calculations using EuclidNN showed that when the feed additive was implemented, the values of the *vCSI2* vector declined (Table 5): *vCSI2*(C2) = 7.5 > *vCSI2*(E2) = 6.6; and *vCSI2*(C3) = 3.3 > *vCSI2*(E3) = 2.6. Hereby, this vector correlates with, and affects the egg production trait. Also, there was a certain relationship between the feed additive intake and feed and water consumption because the correlation coefficients (*R*) of the *vCSI2* vector in relation to these two indicators were positive, though relatively not very high: 0.69 and 0.74, respectively (Table 5).

Discussion

Humankind directly interacts with, and has profound effects on, the multifaceted information environment. Consequently, the relevance of using NNs in these circumstances is increasing. NNs have been already integrated into the lives of the contemporary population, thereby assisting us in solving a large number of problems, especially in the biomedicine area. Modern industrial poultry farming operates with a myriad of objective physiological and genetic data (big data) used to formulate an optimal diet and feeding regime for birds, as well as for the justified application of protective and modulating feed additives. Big data analysis (Gharajeh, 2018; Dall'Alba *et al.*, 2022) allows us to explore the influence of external factors and feed on the health condition and egg production of layers. Based on this analysis outcome, it is possible to develop an optimal strategy for feeding birds in an industrial environment and achieve the highest performance in commercial poultry production. Using the NN approach in this study, we evaluated the increase/decrease in the intensity of physiological/biochemical processes in the body of an agricultural species, specifically in relation to the performance of laying hens by virtue of increasing/decreasing *CSI* index values.

The developed computational EuclidNN, through internal calculations, was instrumental in defining the nature (positive or negative) of the impact that the shungite rock–brown algae feed additive has on the physiological/biochemical processes occurring in the bird's body and, correspondingly, on the performance indicators of laying hens by utilizing the basic blood indices. The latter integrally reflects the hematopoiesis processes resulting in the formation of blood cells such as erythrocytes, leukocytes and platelets (Tietz, 1997). Their production and growth depend on nutritional and vitamin status (Menshikov, 1997; Levchenko *et al.*, 2020; Matveev and Torshkov, 2020; Milevski, 2024; IAKI.RF, 2023). The conversion of feed substrates into nutrients, as well as other mediated physiological/biochemical processes in the hens' body, may have occurred more efficiently when administering the feed additive. For the same reason, the water consumption for keeping birds could also reduce; however, a relatively low correlation of the *CSI2* index with feed and water consumption (R = 0.69 and 0.74, respectively) was probably a consequence of additional accidental losses while keeping the flock of egg layers.

Considering the blood parameters of birds (Tables 1 and 2), we found that they correlated to varying degrees with the *vCSI1* and *vCSI2* vectors. We conclude that the high positive correlation of blood parameters (erythrocyte count and sedimentation rate, pseudoeosinophils, monocytes, and phagocytic index) with the *vCSI1* vector implied that, when using the feed additive, the values of these indicators increased. If other blood parameters (eosinophils, basophils and lymphocytes) correlated with the *vCSI2* vector, this meant that these parameters decreased in response to the feed additive intake. At the same time, it was found that the number of platelets in the blood did not correlate with the *vCSI2* vector (Table 1), suggesting that this blood indicator was not involved in the formation of *vCSI2* vector values and, therefore, may not be measured when diagnosing the health status of birds. In terms of the zootechnical characteristics (Tables 4 and 5), one can note that the feed additive administration most likely caused such a change in physiological/biochemical processes in the bird's body that increased the egg weight while reducing the shell strength. The latter could raise the likelihood of accidental eggshell damage (i.e., cracked, leaking and dirty eggs) during transportation. Consequently, the hens' body responded to the feed additive intake by restructuring internal physiological/biochemical processes, accompanied by an increase/decrease in the birds' blood composition and the performance indicators of laying hens. On the other hand, the NN-based mathematical model was sufficiently sensitive to reveal implicit issues that might affect egg performance and lead to an increased percentage of nonmarket eggs (Table 5).

The use of NN, stochastic fractal-based and similar models has been also assessed in other studies when implementing feed additives for raising egg layers and other farm animals (Nematinia and Abdanan Mehdizadeh, 2018; de Almeida *et al.*, 2020; Ojo *et al.*, 2022; Yang *et al.*, 2023; Buryakov *et al.*, 2023; Siriani *et al.*, 2023). In particular, Buryakov *et al.* (2023) determined the bioconsolidation indices of microorganisms in the intestines of laying hens and estimated the effect of a similar complex feed additive (shungite and *Fucus vesiculosus* seaweed meal) on the self-organization of the microbial-organismal biosystem in the intestines of birds. Calculation of bioconsolidation indices in this and other studies (Kochish *et al.*, 2020b; Buryakov *et al.*, 2023) showed that shungite, in combination with brown algae, can be a promising feed additive for a beneficial effect on the body of layers.

To reduce health risks and financial concerns, decisions regarding poultry production and health status should be made based on objective criteria (de Almeida *et al.*, 2020). Consistent with our study results, de Almeida *et al.* (2020) demonstrated that the use of artificial NNs is a valuable tool to reduce the subjectivity of analysis for predicting and managing poultry flocks and egg production.

The defective eggs we focused upon in our experiments reduce the value of laying hen egg production. Mathematical modelling, as our and other studies (Yang *et al.*, 2023) have shown, can be used to solve problems of improving egg quality and productivity. To this end, Yang *et al.* (2023) developed a convolutional NN-based model to control egg category and weight. To predict egg productivity and freshness, Nematinia and Abdanan Mehdizadeh (2018) used an artificial NN that was trained with the Levenberg-Marquardt algorithm. By implementing research findings like these, the poultry industry can reduce costs and improve productivity.

Ojo *et al.* (2022) stated that the advent of digital technologies has led to significant improvements in various areas. Modern NNs have great potential to intelligently automate current and future poultry management operations to ensure high-quality, low-cost poultry production and manage bird welfare. Siriani *et al.* (2023) tested an NN with a stochastic fractal-based model algorithm to classify the mobility and resting phases of chickens during the rearing process. The stochastic fractal-based model we have developed also allows, by assessing the zootechnical and physiological/biochemical parameters of chicken rearing, to predict their performance and, in this perspective, resistance to diseases.

In general, many models have emerged to date in the field of computer-aided research development that is accompanied by methods for identifying fractals (Meakin, 1999; Dvoryatkina *et al*., 2017; Kochish *et al*., 2023; Yurkovych *et al*., 2023). Analysis and synthesis of biological systems (Chakraverty *et al*., 2023) is a critical element in the task of rising the analysis efficiency of experiments, especially when the sequence of *s* successive elements is considered to be disrupted. Describing complex biological systems requires the use of multidimensional concepts and additional analysis of other time intervals and frequencies of the sample being studied (Pitelinskiy and Shimansky, 2013). Traditional experiments are based on inertial analysis and use flexible linear statistical models (Pyrhönen *et al*., 2024). However, NNs are inherently nonlinear (Ge & Wang, 2002) and do not require knowledge of the correlation between input data and output data (Hsieh, 2000), making them more promising than traditional approaches (Pitelinskiy and Shimansky, 2013). In the current study, multilayer sensitizers (perceptrons; Przybyła-Kasperek and Marfo, 2024) were used to simulate poultry performance and analyze the tolerance of exposure to the complex feed additive based on shungite and algae. However, one should take into account the possible limitations of using closed-loop NNs (Zhu *et al*., 2021), including short-term memory and other potential difficulties.

Collectively, EuclidNN, an NN model we developed using the mathematical stochastic fractal-based method, facilitated studying the effects of the complex feed additive on the immune state of the birds' body, on the intensity of internal physiological/biochemical processes (through their blood parameters) and, ultimately, the performance of layers. As a result, changes in *CSI* index values can be used to estimate changes in the intensity of physiological/biochemical processes in birds' bodies and laying hen productivity.

Conclusion

In the current research, a mathematical stochastic fractal-based model and GNN using this model were developed for the first time, which can be implemented to analyze poultry performance indicators under the influence of the complex feed additive from the mineral shungite and brown algae. Such mycotoxin adsorbents as shungite can have a complex effect on the body of farm animals. Its multifactorial action is associated, first of all, with the removal of negative effects caused by mycotoxins. This study exemplified an NN analysis-assisted evaluation of the effects of the shungite in combination with brown algae on the performance and blood parameters of laying hens in an industrial poultry farm.

We suggest that high bioconsolidation values correspond to increased efficiency of physiological/biochemical processes in the bird's body in response to the feed additive intake. In future, methods for assessing fractal characteristics will be increasingly used for analyzing random processes, developing mathematical models and conducting simulations in biomedicine and in a wide variety of science and technology fields (Pitelinsky and Tyurkin, 2007). The productivity prediction approach using the obtained mathematical model and GNN will be useful in the future improvements of the productive qualities and resilience in poultry.

Traditional poultry studies do not address the one-way ManyToOne relationship of blood parameters with the quantitative and qualitative egg production characteristics of layers, since the qualitative traits are dimensionless and, in a quantitative representation, cannot participate in correlation analysis with the measured characteristics of egg performance. To overcome this obstacle, we created the computational EuclidNN that allowed customizing its calculation procedures to determine the *CSI* values. A distinctive feature of the *CSI* index is that, on the one hand, it characterizes the entire complex of blood parameters of laying hens and, on the other, represents a qualitative or quantitative egg production indicator. Thus, EuclidNN makes it possible to evaluate the complex feed additive influence not only on the quantitative indicators but also on the qualitative egg performance indicators of chickens.

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