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Evaluation of the composition and properties of natural adaptogens for feed additive production

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Abstract

An important aspect of increasing poultry productivity is the development of new feed products. The study aimed to evaluate the composition and properties of natural adaptogens (shungite and fucus crumbles) for the production of feed additives. The composition of shungite and fucus crumbles was investigated. The best survival rate of chickens from the moment of placement and up to 14 weeks of age was observed in the experimental group 1 (97.69%), which is higher than in the control group 1 by 7.05% and in the experimental group 2 by 7.08%. It was found that shungite contains macro and microelements, minerals, and fucus grits contain a significant amount of amino acids: glutamic acid (13.2±0.37 g/100 g of protein), alanine (10.2±0.29 g/100 g of protein), leucine (9.3±0.28 g/100 g of protein), and others. It was demonstrated that the live weight of laying hens in experimental group 2 was 32 g lower than that of control group 1 at 6 weeks, 2 g higher at 12 weeks, and 34 g higher at 14 weeks. The study's findings revealed that when transferring young stock to market shops at 14 weeks of age, the uniformity of hens in the experimental group 1 was 6.7% higher than in the control group, and 13.5% higher in the experimental group 2. The digestive function is performed by several phylum of bacteria inhabiting the gastrointestinal tract, the majority of which are bacteria of the phylum Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria. The proportion of microorganisms of the Actinobacteria phylum increases in the experimental groups: 1.45 times in the group receiving shungite-based feed additives compared to the control group, 2.72 times in the group receiving brown algae-based feed additives compared to the control group. The positive effect of fucus crumbles on the increase in the number of normoflora of ceca was confirmed. The results of the study will be used in poultry husbandry to improve egg weight and quality, the content of crude protein, carotenoids, and vitamin A in egg mass, the digestibility of amino acids lysine and methionine, hemoglobin content, immunity, and the incidence of fatty deposits in the liver.

Keywords: Shungite, Fucus vesiculosus, Chickens, Productivity, Immune status.

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1. Introduction

Poultry production is one of the fastest growing branches of agriculture [1]. The widespread of poultry production stems primarily from the high profitability of egg and meat production in comparison to other animals, as well as the high nutritional value of these products [2,3]. Antibiotics have become widely used as growth stimulants [4] feed additives to prevent infectious diseases [5,6] and other chemicals, all of which have significant disadvantages. All these substances remain in the final poultry meat products [7,8] which poses a serious threat to human health. Antibiotic residues in poultry products promote the synthesis of numerous pathogenic bacteria and the spread of antibiotic-resistant bacteria. Furthermore, there is an increased risk of antibiotic-resistant pathogens spreading in uncontrolled environments, and the use of artificial animal breeding has an impact on human health, the economy, and national security [9,10]. Another important aspect of improving poultry productivity is the development of new feed products suitable for birds reared with low nutrient components in the diet [11]. Using algae could prove to be a promising direction in this field [12–14]. The most advanced approach in this regard is the use of individual algae (e.g., spirulina, chlorella, spirogylla) in animal feed [15]. Their widespread use in human and animal diets is due to their low production costs, ease of cultivation, and rich chemical composition [16,17]. However, there are complex algae in coastal areas of Russia, especially in the Arctic Ocean, the White Sea, and other seas [14]. Brown algae are marine organisms with complex organization. They include 1500 species assigned to 265 genera including *Laminaria*, *Sargassum*, *Cystozella*, and *Fucus albicans* [18]. Many authors believe that brown algae have a balanced nutrient composition, contain a significant amount of amino acids, including essential amino acids, and contain complex biologically active substances, chelate compounds, which improve the longevity and productivity of plants and animals of various species [19,20]. However, these studies focused primarily on algae growing in temperate climates, primarily in coastal Japan [21], the European Union [17], the United States, and the United Kingdom [22]. The main area of Fucus algae in Russia is on the Arctic and Atlantic coasts, which accounts for their unique chemical composition and properties [8, 23].

Modern sorbents based on shungite rock with particles up to 1 mm in size, commercially mined at the Zazhoginskoye deposit in the Republic of Karelia, are one of the alternative products [24]. Shungite is a natural antioxidant that boosts human immunity and prevents many serious diseases. It is undeniably interesting for agriculture, cattle breeding, and poultry breeding. Still, not much is known about the potential of shungite, and not much research has been done on it even in the area of poultry production. Shungite application in the composition of mixed fodder for laying hens requires scientific and industrial-economic substantiation, as well as research into its effect on zootechnical, biochemical parameters, feed digestibility, and mineral metabolism in poultry [24]. Consequently, this study aimed to evaluate the composition and properties of natural adaptogens (shungite and fucus crumbles) for the production of feed additives.

2. Materials and methods

Shungite from the Zazhoginsky deposit (Karbon-Shungit, Petrozavodsk, Russia) with a particle size of 0-15 mm and a moisture content of up to 10% was used as the raw material for the production of mineral feed additive. The carbon (C) content of the shungite was 25-35%. *Fucus vesiculosus* was collected from the White Sea coast (Belomorsk, Republic of Karelia, Russia) and pre-dried to 15-18% moisture content for the production of brown algae feed additives.

- Study of the shungite and fucus crumbles composition
- Studies of the chemical composition of fucus crumbles were performed according to the method described in [25].

When investigating the safety of the developed products positioned as poultry feed additives, they were tested for the presence of toxic substances. The amount of nitrite was determined using the photometric method. The determination of benz(a)pyrene was also performed on these products, using the methodology described in [26]. Organophosphorus compounds (OPCs) (diazinon, dimethoate, melathion, parathion-methyl, pyrimiphos-methyl, phosalone, phosmet, chlorpyrifos, fenitrothion, etc.) were determined. The determination of various compounds of this group was performed according to [26]. To determine the toxicity of feed additives, organochlorine compounds (OCCs) (HCCH and sum of isomers, DDT, and metabolites, hexachlorobenzene, aldrin, heptachlor, endrin, endosulfan, etc.) were determined. The determination of the sum of HCCH and isomers was performed according to [25]. DDT and its metabolites were determined according to [27].

The contents of arsenic, cadmium, lead, mercury strontium-90, and cesium-137 were determined according to [28]. The total toxicity of feeds was determined: complete analysis (express method and arbitration method) according to the method described in [25]. Experiment design is presented in Table 1.Technological parameters of maintenance of rearing flocks during the experiment in all groups corresponded to the parameters established at the enterprise for the given cross and age. The parameters of keeping rearing flocks are presented in Tables 2-3. The following indicators were considered during the experiment: physical and chemical analysis of feed (at the arrival of each batch); clinical and physiological condition of poultry (daily); live weight and uniformity of chickens (weekly); flock survival and waste reasons for losses (daily by groups); feed consumption (daily by groups). The contents of cecum of birds were sampled and the intestinal microbiota was analyzed using high-throughput sequencing on the genetic analyzer GTZ G08 / Genetic Analyzer G08 (Gennye Tekhnologii Zdorovya, Moscow, Russia) to reveal the mechanisms of action of natural adaptogens from the mineral shungite and fucus crumbles. Following the completion of the feeding experiments, tissue samples were collected and preserved for subsequent gene expression analysis. Tissues from the uterus, oviduct, and intestine were sampled in control and experimental samples of hens (6 hens each) to determine the specific effect of the supplement on productivity components. Because gene expression studies are done at the mRNA level, tissue selection and fixation were done within 15-20 minutes of slaughtering to reduce mRNA loss in the samples and potential errors in the overall analysis. Tissue extraction was performed with clean instruments pretreated with RNase ZAP™ to remove RNases from the surface.

The size of each sample did not exceed 5 mm in any one measurement, with a mass not exceeding 100 mg. Immediately after extraction, samples were placed in a 1.5-2.0 mL eppendorf with pre-infused fixative (RNAlater). Samples in RNAlater fixative can be transported without special means. The tissue samples were stored in a freezer at -20 °C. A total of 54 samples were collected for each variation of the feeding experiment. The IL8 gene – interleukin 8 gene – was used as a resistance marker gene. This gene encodes a protein of the cytokine class and a marker of chronic and acute inflammation, causing directed migration of neutrophils and lymphocytes to the site of inflammation. MEPE (OC-116), a bone and shell mineralization protein, was used as a marker gene for productivity. Gene expression was determined using the polymerase chain reaction (PCR) method on a real-time PCR instrument MA-6000/ qPCR MA-6000 (Gennye Tekhnologii Zdorovya, Moscow, Russia). Statistical processing of the research results consisted in finding significant differences between the mean values in the samples. The mean values for the groups, the errors of the mean in body mass, weight and relative weight of internal organs, morphometric indicators, the statistical significance of differences between the group of test systems receiving TS and the control group were determined, indicating, if there are differences, the values of the statistical criterion, the number of degrees of freedom (df) and the probability of forecast error (p). Outliers were detected according to the Grubbs' criterion [29, 30]. The dynamics of body mass was assessed by the indicator of the relative increase in body mass according to Brody's equation [30].

$$K = \frac{(W_t - W_0)}{0.5 \times (W_t - W_0)} \times 100\ \%;$$

where K – relative growth (%) for a certain period; W_t – weight at a given age, g; W_0 – initial weight, g.

The relative mass of organs (relative to body weight) was calculated according to the equation:

$$M_{\text{т}} = m/M \times 100\%;$$

where $M_{\text{т}}$ – relative organ mass by body mass, %; m – organ mass, g; M – bodyweight of the bird, g.

3. Results and Discussions

The resulting feed additive samples were tested for safety. The results are presented in Table 4. Table 4 data demonstrates that all samples were safe. The results of determining the composition of mineral additive from shungite are presented in Table 5. The nutritive value of shungite was determined to be zero. The functionality of shungite is associated with the mineral's sorption activity in relation to toxic elements, including mycotoxins, as evidenced by data from the literature [25-27].The chemical composition of fucus crumbles is summarized in Tables 6-7. Table data reveal that fucus crumbles are rich in carbohydrates (29.2±0.33%), sulfur-containing polysaccharide fucoidan (2.73±0.16%), and polyphenols (0.6±0.021%). It also contains proteins, lipids, dietary fiber, alginates, and iodine. The data (Table 7) suggest that glutamic acid (13.2±0.37 g/100 g of protein), alanine (10.2±0.29 g/100 g of protein), and leucine (9.3±0.28 g/100 g of protein) were the most abundant in fucus crumbles. Tryptophan (1.0±0.03 g/100 g of protein) and cysteine (1.5±0.04 g/100 g of protein) were least abundant. The live weight parameters considered in our studies are presented in Table 8. When analyzing the data in Table 8, it was found that the greatest dynamics of live weight was recorded for the experimental group 1 that recieved shungite. Along with the average live weight indicator, uniformity is an indicator of the flock's normal development during the rearing period. The results of the uniformity study are presented in Table 9. Our findings revealed that at 14 weeks of age, when transferring young stock to commercial shops, the uniformity of hens in the experimental group 1 was 6.7% higher than in the control group, and 13.5% higher in the experimental group 2. Zootechnical parameters of laying hens after 14 days of feeding are presented in Table 10. The data in Table 10 show that the experimental group 1 had the highest livability of chickens from planting to 14 weeks of age - 97.69%, which is higher than the control group 1 (90.64%) and experimental group 2 (90.61%). The dynamics of young stock losses is presented in Table 11.

An examination of the dynamics of chicken losses by age reveals an increase in this indicator beginning in the sixth week of rearing, which is associated with the acute postvaccinal reaction to infectious laryngotracheitis of birds, followed by complications in the form of chronic respiratory diseases and, finally, with the layering of secondary bacterial infection. The losses were observed in all groups, but they were significantly lower in experimental group 1 against the background of shungite introduction. An important factor in growing young chickens is the consumption of compound feed (Table 12). During the experiment, the highest feed intake was observed in the experimental group 1 – 47.32 g/head, which is 2.67% higher than in the control group. In the experimental group 2, on the contrary, feed consumption was slightly higher than the control. The sharpest decrease in appetite in birds and, as a consequence, lower feed intake was observed during the period of postvaccinal stress at 5 and 6 weeks of rearing. Thus, the adaptogenic effect of the mineral shungite was confirmed. Shungite can relieve stress against the background of poultry vaccinations.The lowest feed consumption per 1 cwt. of live weight gain was found in experimental groups 1 and 2 (3.6 and 3.55) cwt. This indicates a higher coefficient of utilization of feed nutrients by chickens of the experimental groups. The results of the study of the gut microbiota of young laying hens using the NGS method are presented in Table 13.

Genes of poultry adaptation to heat stress are determined against the background of evaluation of expression of model genes of productivity and resistance. The genes presented in Table 14 were selected for further studies. Mineral shungite and brown algae of the genus *Fucus*. Raw materials for feed additives were obtained from the Republic of Karelia. Raw material preparation included milling and classification to fractions with particle sizes ranging from 0.2 to 0.8 mm. This range complies with the regulations for feeds and feed additives.The additive samples were tested for toxicity using physicochemical and toxicological methods. It was found that there was no toxicity in the feed additives studied. It was found that all normalized toxic elements in feed additives from shungite and algae were within MPC. The safety of feed additives and veterinary preparations was determined by biotesting. All samples were sasfe. According to research (Table 4), the amount of nitrite in feed additives based on shungite was less than 0.05 mg/kg, and the amount of nitrite in feed additives based on brown algae was also less than 0.05 mg/kg, both of which are less than the maximum permissible values for mixed fodder and feed raw materials. The benz(a)pyrene concentration in shungite-based feed additives was 0.00070.0003 mg/kg, while seaweed-based feed additives had a concentration of 0.00030.0001 mg/kg, with an acceptable range of 0.0001-0.1 mg/kg for food products. The content of HCCH sum and isomers in shungite-based and fucus-based feed additives was less than 0.01 mg/kg. These feed additives contained less than 0.005 mg/kg of trichlorometaphos, less than 0.01 mg/kg of rogor, less than 0.01 mg/kg of malathion, less than 0.002 mg/kg of metaphos, and less than 0.005 mg/kg of bazudine. Brown algae-based feed additives were found to have trichlorometaphos content less than 0.005 mg/kg, hornwort content less than 0.01 mg/kg, malathion content less than 0.01 mg/kg, metaphos content less than 0.002 mg/kg, and bazudine content less than 0.005 mg/kg (Table 4). These values are below the maximum permissible concentrations. The content of DDT and its metabolites in shungite-based and seaweed-based feed additives was less than 0.01 mg/kg. These values were significantly below the maximum permissible concentrations for feeds and feed additives. Arsenic concentrations in feed additives based on shungite were 0.03 mg/kg, while arsenic concentrations in feed additives based on seaweed were 0.01 mg/kg, both of which were less than the maximum permissible values (0.1 mg/kg). Cadmium concentration in shungite-based and brown algae-based feed additives was less than 0.1 mg/kg. The concentration of lead in shungite-based feed additives was 0.55 mg/kg. The concentration of lead in algae-based feed additives was 0.45 mg/kg. The concentration of mercury in shungite-based feed additives was 0.026 mg/kg. The concentration of mercury in fucus-based feed additives was less than 0.0025 mg/kg. Strontium 90 concentration in shungite-based feed additives was less than 18.1 Bq/kg. The concentration of strontium 90 in fucus-based feed additives was less than 22.9 Bq/kg. The concentration of caesium 137 in shungite-based feed additives was less than 12.8 Bq/kg. The concentration of caesium 137 in seaweed extract-based feed additives was less than 14.2 Bq/kg. According to the results of the experiments (Table 4), feed additives based on shungite and seaweed extracts are non-toxic when used in feed at concentrations of at least 10 kg per ton, which is significantly higher than the planned concentrations in the experiment. Only when the live weight of young poultry in the process of growing corresponds to the reference values according to a specific age period is it possible to fully realize all biological characteristics of poultry (Table 8). The most representative periods in the rearing process of young birds are 6 and 12 weeks of life. The organs of the digestive and immune systems develop intensively before 6 weeks of age. Stressed birds at this time may experience issues with digestion and nutrient absorption during the housing period, which could lead to immunosuppression, or weakened immunity. Furthermore, until 12 weeks of age, muscle mass, backbone, and plumage develop rapidly, which is critical for achieving high levels of productivity and good eggshell quality.

Live weight of hens of the experimental group 2 at 6 weeks was 32 g (6.85%) lower than that of the control group 1, 2 g (0.17%) higher at 12 weeks and 34 g (2.63%) higher at 14 weeks (Table 8). Almost throughout the whole period of rearing, the highest live weight was observed in hens of the experimental group 1. The superiority of this index at 6, 12 and 14 weeks of age relative to the control group 1 was 53 g (12.3%), 91 g (8.01%), and 73 g (5.67%), respectively (Table 8). Along with the average live weight indicator, the indicator of normal development of the flock during the rearing period is uniformity (Tables 9, 10). The study's findings revealed that when transferring young stock to production shops at 14 weeks of age, the uniformity of hens in the experimental group 1 was 6.7% higher than in the control group, and 13.5% higher in the experimental group 2. The experimental group 1 had the highest level of chicken livability from the time of stocking until 14 weeks of age - 97.69%, which is 7.05% higher than the control group 1 and 7.08% higher than the experimental group 2 (Tables 9, 10). Post-mortem examination of dead birds revealed that the causes of death were nearly identical in all groups. An increased number of chickens died from chronic respiratory diseases (23.3-35.62%), respiratory diseases (9.95-19.8%), and colibacillosis (7.5-14.9%). It should be noted that the incidence of chronic respiratory diseases was -1.95% in experimental group 1 (on shungite background) (Tables 10, 11). Based on the findings, it can be assumed that shungite administration improves the efficacy of the vaccinations. The addition of fucus crumbles to the diets of young birds had no effect on bird livability when compared to the control variant.

The chicken crop (Table 10) is the most informative indicator for characterizing the quantity and quality of marketable young stock based on growing results. The highest index of young chicken crop was obtained in the experimental group 1, on the background of shungite introduction - 96.64%, which not only exceeds the indicators of other groups, but also the reference values - by 0.44%. The lowest feed costs per 1 cwt of live weight gain were found in experimental groups 1 and 2 (3.6 cwt and 3.55 cwt). This indicates a higher coefficient of feed nutrient utilization by chickens of the experimental groups (Table 12). The contents of the blind intestinal tracts of poultry were sampled to reveal the mechanisms of action of natural adaptogens derived from the minerals shungite and fucus crumbles. The gut microbiota was analyzed using high-throughput sequencing. The results of the study are summarized in Table 13. It should be noted that several phyla of bacteria inhabiting the gastrointestinal tract perform the function of digestion, the majority of which are bacteria of the phyla Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria. According to the data in Table 14, the proportion of microorganisms of the phylum Actinobacteria (at the expense of the genus Bifidobacteriales) increases in the experimental groups: 45.45% (1.45 times) in the group receiving shungite-based feed additives compared to the control group, and 172.01% (2.72 times) in the group receiving brown algae-based feed additives compared to the control group. At the same time, the proportion of microorganisms of this phylum in the group receiving algal extract feed additives was 87% (1.87 times) higher than in the group receiving shungite feed additives.

Members of the above phylum are involved in the decomposition of organic matter such as cellulose and chitin, and thus participate in the organic matter cycle and the carbon cycle. The proportion of Bacteroidetes phylum bacteria increased in the group receiving shungite-based feed additives by 12.23% (1.12 times) compared to the control group. In the group receiving algae-based feed additives, the number of microorganisms decreased by 439.49% (5.39 times) compared to the control group. At the same time, the number of bacteria in the group receiving shungite as a feed additive was 505.50% higher (6.06 times higher) than in the group receiving fucus crumbles as a feed additive. The proportion of representatives of phylum Deferribacteres was higher by 70.16% (1.76 times) compared to the control group in the group receiving shungite as a feed additive. The group that received fucus algae as a feed additive showed no growth of bacteria of this phylum. The proportion of representatives of Firmicutes phylum was higher in the experimental group receiving shungite as a feed additive by 50.37% (1.5 times) compared to the control group. When birds were given fucus crumbles, the proportion of members of this phylum increased by 132.57% (2.32 times) when compared to the control group. When birds were given fucus croup, the proportion of members of this phylum increased by 132.57% (2.32 times) when compared to the control group. The proportion of bacteria of the genus *Lactobacillales* was less in the group that received shungite as a feed additive by

365.40% (4.65 times) compared to the control group. The proportion of bacteria of the genus *Lactobacillales* was less in the group that received shungite as a feed additive by 365.40% (4.65 times) compared to the control group. There was a 60.56% (1.6-fold) decrease in the proportion of bacteria of the genus *Clostridiales* in the group with shungite compared to the control group. The proportion of microorganisms of the genus *Clostridiales* was higher by 106.67% (2.07 times) in the group receiving brown algae as a feed additive compared to the control. The proportion of bacteria of the Ruminococcaceae family was lower by 41.20% (1.41 times) in the group receiving shungite as a feed additive. The positive effect of fucus crumbles on the abundance of Ruminococcaceae was found to be higher by 59.47% (1.59 times) compared to the control. When algal extracts were used as a feed additive, the proportion of bacteria in the Ruminococcaceae family was 125.18% higher (2.25 times higher) than when shungite was used. It should be noted that the proportion of *Proteobacteria phylum* microorganisms was higher by 137.66% (2.38 times) in the group receiving shungite as a feed additive compared to the control group. At the same time, the proportion of Proteobacteria phylum bacteria decreased by 719.62% (8.19 times) on the background of fucus crumbles intruduction, compared to the control group. The proportion of microorganisms in the group that received shungite as a feed additive was 19.48 times higher than in the group that received seaweed extracts as a feed additive. The proportion of *Tenericutes phylum* was higher in the group receiving shungite as feed supplement by 225.97% (3.26 times) compared to the control group. No growth of Tenericutes phylum bacteria was observed in the group receiving shungite as a feed additive. The proportion of Cyanobacteria phylum bacteria was higher by 103.87% (2.04 times) in the group receiving shungite as a feed supplement compared to the control group. The proportion of *Cyanobacteria phylum* microorganisms was 24.26 times higher in the group receiving seaweed extracts as feed supplement compared to the control group. The proportion of Cyanobacteria phylum microorganisms was 11.90 times higher in the group receiving seaweed extracts as feed supplement compared to the group receiving shungite as feed supplement. The proportion of Bacteroidia class bacteria was higher by 9.61% (1.1-fold) in the group receiving shungite as a feed additive compared to the control group. The proportion of microorganisms of Bacteroidia class was 8.22 times higher in the group receiving seaweed extracts as feed additive compared to the control group. The proportion of microorganisms of Bacteroidia class was 9.01 times higher in the group receiving seaweed extracts as feed supplement compared to the group receiving shungite as feed additive. The proportion of unclassifiable bacteria was not significantly different between the control group and the group receiving shungite as a feed additive. The group receiving seaweed extracts as feed additive had a higher proportion of unclassifiable bacteria by 43.39% (1.43 times) compared to the control group. The group that received shungite as a feed additive grew bacteria from the phyla Fusobacteria and Synergistetes, whereas the other groups did not grow bacteria from these phyla. At the same time, representatives of the phylum Synergistetes are opportunistic microflora. There are known results of studies by other authors in the field of applying shungite and fucus crumbles in the feeding of laying hens [28]. According to the study [28], to solve the problem of using shungite in poultry feeding, four groups of 140-day-old laying hens, each with 20 heads, were chosen using the pair-analogs principle. The first and second groups of birds were control, the third and fourth groups were experimental. The first control group received the basic diet and the second control group received fusariotoxin contaminated compound feed. In compound feeds for poultry of the third experimental group, infected with fusariotoxins, 1% of ground shungite was added. Poultry in the fourth experimental group wer fed a diet containing 98.5% fusariotoxic compound feed and 1.5% ground shungite (grinding 1.5-2 mm). The duration of the experiments was 6 months. Before starting the experiments, the chemical composition of fucus algae crumbles was studied. It was found that the dry matter of fucus grits contains (%): crude protein 7.82, crude fiber 8-10 %, crude ash 17-25 %, non-extractive substances - 36-45 %. The total amino acid content is 53.12 g/kg, high content of glutamic acid 10.11 g/kg and asparagic acid 8.81 g/kg was observed [28].

Inclusion of fucus crumbles in the diet had a positive effect on the livability of chickens. The lowest livability of chickens was in the control and second experimental groups (96 and 95.8 %). In experimental groups 6 and 7 it was higher than in the control group by 2.2 and 1.5 %. Live weight of 490-day-old chickens did not differ significantly between groups. The maximum egg production per initial laying hen - 149.9 eggs - was obtained in experimental group 6, which is 10.6 eggs higher than the control group and 4.2-11.2 eggs higher than the experimental groups. Egg mass from laying hens in experimental group 6 was 7% higher than in the control group, and by 3.4-7.9% higher than in the other experimental groups. Based on the conducted research it was found that the use of shungite in the feeding of laying hens was reflected in the increase in the livability of birds. Thus, for 180 days of experimentation, it amounted to 100% in experimental groups 3 and 5, 97.5 and 95.5% in experimental groups 2 and 4, and 92.5% in the control group [28]. Thus, for 180 days of experimentation, it amounted to 100% in experimental groups 3 and 5, 97.5 and 95.5% in experimental groups 2 and 4, and 92.5% in the control group [28]. The use of shungite increased the intensity of egg production of hens of the experimental group by 11.2 % compared to the control group. The experimental group's productivity remained at its peak throughout the duration of the study. The addition of shungite improved shell quality and reduced the percentage of check and egg breakage. After 40 days of shungite inclusion, the thickness of egg shells in experimental groups increased by 0.02 mm, or 5.6%, compared to the control group; after 120 days, the difference was 0.019 mm, or 5.2%. The number of rejects (breakage and check) in the experimental groups was 26.8 % lower than in the control group. Feed costs per 10 eggs and 1 kg of egg mass in the experimental group were 13.1-16.0% lower than in the control group, at 1.40 and 2.25 kg, respectively [28]. The authors Trukhachev et al. studied the effect of a shungite-based feed additive on young chickens of the Iza Hubbart F-15 cross [27]. Shungite use had a significant immunostimulating effect, which reduced culling of poultry, increased its livability, productivity, and immune response of the organism when planning vaccination. The adsorption properties of the mineral shungite, a natural adsorbent, were investigated in [24].

Table 1: Experiment design

Group	Number of heads	Feeding
Control 1	55900	Main diet (MD) – traditional compound feed for rearing flocks
Experimental 2 (building No. 8)	56200	Special diet (SD) – compound feed for rearing flocks with addition of shungite (1 kg/t of mixed fodder)
Experimental 3 (building No. 10)	58000	Main diet (MD) – traditional compound feed for rearing flocks with addition of fucus crumbles (1 kg/t feed)

Table 2: Water consumption, mL/head

Age, weeks	Consumption water (recommendations for the cross)	Control 1	Experimental 1	Experimental 2 (building No.9)
1	21-30	15.0	19.0	16.0
2	26-42	25.0	28.0	27.0
3	35-50	32.0	48.0	35.0
4	41-58	47.0	51.0	47.0
5	51-72	56.0	64.0	60.0
6	57-80	58.0	67.0	62.0
7	57-80	65.0	79.0	73.0
8	68-94	78.0	87.0	78.0
9	74-106	93.0	98.0	92.0
10	78-112	99.0	100.0	99.0
11	87-124	103.0	108.0	103.0
12	93-132	113.0	111.0	113.0
13	101-142	117.0	104.0	130.0
14	105-148	128.0	112.0	124.0

Table 3: Temperature and humidity conditions

Group	Weeks													
										0	1	2	3	4
Temperature conditions, °C														
Reference values	4-32	2-30	0-28	8-26	6-23	3-21	1	1	1	1	1	1	1	1
Control 1	3-32	1.5- 9.2	9-27	7-26	4.1- 4.0	3.6- 3.0	2.2	2.3	2.9	2.7	3.3	3.3	3.3	2.8
Experimental 1	2.8- 9.6	9.6- 8.9	8.9- 7.1	7.1- 6.3	5.9- 4.4	4.2- 3.3	3.3	3.5	2.5	2.4	2.3	2.3	2.4	2.3
Experimental 2	3.3- 2.1	2.1- 0.0	0.0- 8.6	8.2- 6.1	6.0- 4.7	4.7- 2.5	2.0	1.9	1.6	1.5	1.2	1.2	1.2	1.3
Humidity conditions, % (reference values 40-50%)														
Control 1	0.0	7.7	4.6	4.0	0.8	1.3	1.0	0.0	1.5	9.0	0.6	1.0	0.0	0.1
Experimental 1	0.8	0.0	0.0	5.2	5.5	6.5	8.0	1.7	3.3	0.3	0.7	1.7	2.6	8.2
Experimental 2	8.4	7.1	2.5	4.4	4.4	5.5	6.1	5.3	4.8	6.1	5.8	6.8	5.7	4.4

Table 4: Determination of the safety of natural adaptogens, in accordance with the requirements for feed and feed additives

Type of study	Indicators	Shungite	Fucus crumbles
1. Testing for the presence of toxic substances	1. Determination of nitrite (compound feed, compound feed raw materials)	less than 0.05 mg/kg	less than 0.05 mg/kg
	2. Determination of benz(a)pyrene (food products)	0.0007±0.0003 mg/kg	0.0003±0.0001 mg/kg
	3. Determination of organophosphorus compounds (OPCs) (diazinon, dimethoate, melathion, parathion-methyl, pyrimiphos-methyl, phosalone, phosmet, chlorpyrifos, fenitrothion, etc.).		
	- trichloromethaphos	0.005 mg/kg	<0.005 mg/kg
	- rogor	<0.01 mg/kg	<0.01 mg/kg
	- malathion	<0.01 mg/kg	<0.01 mg/kg
	- methaphos	<0.002 mg/kg	<0.002 mg/kg
	- bazudin	<0.005 mg/kg	<0.005 mg/kg
	4. Determination of organochlorine compounds (OCCs) (HCCH and sum of isomers, DDT and metabolites, hexachlorobenzene, aldrin, heptachlor, endrin, endosulfan, etc.):		
	HCCH and isomers, sum	less than 0.01 mg/kg	less than 0.01 mg/kg
	DDT and its metabolites	less than 0.01 mg/kg	less than 0.01 mg/kg
	5. Determination of arsenic (food products)	0.03 mg/kg	0.01 mg/kg
2. Toxicological studies	Cadmium	less than 0.1 mg/kg	less than 0.1 mg/kg
	Lead	0.55 mg/kg	0.45 mg/kg
	Mercury	0.026 mg/kg	less than 0.0025 mg/kg
	Strontium 90	less than 18.1 Бк/кг	less than 22.9 Бк/кг
	Cesium 137	less than 12.8 Бк/кг	less than 14.2 Бк/кг
	Determination of total feed toxicity: complete analysis (rapid method and arbitration method)	Non-toxic at rate of introduction into feed according to label	Non-toxic at a feed introduction rate of 10 kg/ton of feed

Table 5: Chemical composition of mineral additive from shungite

Main components	Content, % wt.
SiO_2	62.69±1.74
Al_2O_3	3.46±0.08
Fe_2O_3	2.43±0.05
MnO	<0.01
CaO	0.17±0.002
MgO	0.96±0.005
Na_2O	<0.3
K_2O	1.05±0.03
P_2O_5	0.06±0.009
SO_3 / SC	0.72/0.49
$C_{total.}$	28.1±0.16
Cu	0.0058

Table 6: Chemical composition of fucus crumbles

Indicator	Content, %
Protein	21.8±1.68
Lipids	13.5±0.58
Carbohydrates	29.2±0.33
Ash	19.7±0.22
Fibers	7.1±0.10
Moisture content	8.7±0.25
Mass fraction of iodine	0.1± 0.02
Mass fraction of alginates	33.3± 1.12
Mass fraction of fucoidan	2.73± 0.16
Mass fraction of polyphenols	0.6± 0.021

Table 7: Amino acid composition of fucus crumbles

Amino acids	Amino acid content, g/100 g of protein
Alanine	10.2±0.29
Arginine	6.0 ±0.18
Aspartic acid	8.6±0.27
Cysteine	1.5±0.04
Glutamic acid	13.2±0.37
Glycine	6.5±0.19
Histidine	3.2±0.06
Isoleucine	3.8±0.11
Leucine	9.3±0.28
Lysine	6.3±0.19
Methionine	2.1±0.06
Phenylalanine	6.9±0.21
Phenylalanine	4.9±0.15
Serine	2.8±0.09
Threonine	5.2±0.15
Tryptophan	1.0±0.03
Tyrosine	7.2±0.20
Valine	7.3±0.21

Table 8: Dynamics of live weight of young chickens (g)

Age, weeks	Reference values	Group		
		Control 1	Experimental + shungite	Experimental 2 + Fucus crumbles
1	70	71	74	66
2	125	134	140	128
3	190	203	220	200
4	265	299	297	284
5	360	376	406	380
6	460	429	482	435
7	560	531	588	531
8	670	677	730	669
9	780	816	868	814
10	890	938	1012	960
11	990	1042	1134	1080
12	1080	1136	1227	1174
13	1160	1195	1322	1248
14	1230	1288	1361	1327

Table 9: Dynamics of uniformity %

Age, weeks	Reference values	Group		
		Control 1	Experimental 1 (+shungite)	Experimental 2 (+Fucus crumbles)
1	85.0	77.5	73.5	69.5
2	85.0	87	75	78.5
3	85.0	75	85.5	82
4	80.0	81.0	85.4	88.0
5	80.0	76.4	89.3	84.0
6	80.0	64.5	70.0	69.5
7	85.0	67.0	80.0	62.5
8	85.0	76.5	85.5	65.5
9	85.0	78.5	82.5	70.0
10	85.0	83.5	88.5	77.0
11	85.0	86.5	92.5	80.0
12	85.0	84.5	90.5	79.0
13	85.0	88.5	87.5	76.5
14	85.0	84.3	91.0	95.7

Table 10: Zootechnical indicators and comparison of feeding rations

Indicator	Control 1	Experimental 1	Experimental 2
Initial data			
Hatching date	07-08.08	14-15.08	28-29.08
Numbers of head	55900	56200	58000
Parental age	55	56	56-58
% of hatching: reference aactual	 78 77.6-72.5	 78 75.9-74.9	 75-80 78.7-80.1
Day-old chicken weight, g	38.5-38.6	36.3-38.4	38.3-39.0
Average weight of 1 head at admission, g	37-38	38-40	36-38
Uniformity at admission, %	88.0-92.0	84.0-86.0	86.0-91.0
Zootechnical indicators and comparison of feeding rations			
Feeding days	5355835	5560741	5612319
Live weight at the age of 14 weeks, g (reference value 1230 g)	1288	1361	1293
Average daily weight gain, g (reference value 11.65 g)	12.21	13.14	12.32
Gross live weight gain for the period of experiment, kg	65411	73071	69163
Uniformity, % (reference value 85.0%)	84.3	91.0	95.2
Mortality, heads	5231	1299	5447
Livability, % (reference value 97.9%)	90.64	97.69	90.61
Culling, %	6.03	1.06	5.01
Chicken crop, % (reference value 96.2%)	84.62	96.64	85.61
Feed consumption: per 1 head, g (reference value 49.02 g) per 1 cwt. of gain, cwt. (reference value 4.21 g)	 46.09 3.77	 47.32 3.60	 46.54 3.78
Transferred to the production shop /building No.	2..6	4..17	3.11
Heads transfered	47299	54307	49647
% of heads of the studied groups in the production shop	71.7	82.3	77.8
Eggs before transfer, pcs (reference value 5.2)	1.07	1.10	1.54

Table 11: Dynamics of young stock losses, %

Age, weeks	Group		
	Control 1	Experimental 1	Experimental 2
1	0.65	0.64	1.21
2	0.05	0.10	0.13
3	0.04	0.02	0.08
4	0.01	0.04	0.02
5	0.07	0.01	0.05
6	0.72	0.09	0.10
7	0.58	0.08	0.19
8	0,75	0,13	0,63
9	1,02	0,09	0,63
10	0,68	0,08	0,65
11	0,78	0,16	0,93
12	1,08	0,46	1,20
13	1,74	0,32	1,64
14	1,33	0,10	1,53

Table 12: Feed consumption, g/head

Age, weeks	Group		
	Control 1	Experimental 1	Experimental 2
1	16.7	16.4	15.9
2	21	20.3	20
3	25.2	22.8	26.3
4	31.1	27.2	29.9
5	32.8	39.6	38.2
6	33	39.2	37.6
7	41.3	42	48.5
8	54.3	52.2	45.6
9	62.7	59	58.0
10	68.4	63.7	70.4
11	68.1	69.7	71.0
12	59	73.2	70.8
13	69.8	73.6	74.6
14	75.5	76.8	70.8
Period after transfering	46.09	47.32	46.54
+/-	-2.93	-1.7	-2.48

Table 13: Analysis of intestinal microbiota of young laying hens

Variants		control	+ shungite	+ brown algae
Phylum	*Actinobacteria*	0.154	0.224	0.419
Genus	*Bifidobacteriales*	0.154	0.224	0.419
Phylum	*Bacteroidetes*	58.54	65.703	10.851
Phylum	*Chlamydiae*	0	0	0
Phylum	*Chloroflexi*	0	0	0
Phylum	*Deferribacteres*	0.124	0.211	0
Phylum	*Elusimicrobia*	0	0	0
Phylum	*Firmicutes*	31.989	21.273	74.396
Genus	*Lactobacillales*	2.704	0.581	12.972
Genus	*Clostridiales*	28.52	17.763	58.942
Family	*Ruminococcaceae*	11.367	8.05	18.127
Genus	*Selenomonadales*	0	0	0
Phylum	*Fusobacteria*	0	1.044	0
Phylum	*Gemmatimonadetes*	0	0	0
Phylum	*Lentisphaerae*	0	0	0
Phylum	*Proteobacteria*	2.172	5.162	0.265
Family	*Enterobacteriacea*	0	0	0
Phylum	*Spirochaetes*	0.378	0	0
Phylum	*Synergistetes*	0	0.152	0
Phylum	*Tenericutes*	0.154	0	0.502
Family	*Mycoplasmataceae*	0	0	0
Phylum	*Verrucomicrobia*	0	0	0
Phylum	*Cyanobacteria*	0.181	0.369	4.392
Class	*Bacteroidia*	51.936	56.928	6.318
Unclassified		6.31	6.385	9.048

The adsorption properties of the mineral shungite, a natural adsorbent, were investigated in [24]. The goal of this study was to provide scientific and industrial-economic support for the use of shungite in compound feed for laying hens, as well as to investigate its impact on zootechnical, biochemical, feed digestibility, and mineral metabolism state in poultry. The following goals were set in order to complete the experiment:

- Evaluate the effect of shungite on livability, live weight, egg production, and egg quality.
- Determine the effect of shungite on ration quality, conversion, and digestibility of feed nutrients.

It was found that shungite had an intensifying effect of egg production, the intensity of egg laying led by groups. Weight, density, shell thickness, Haugh unit, presence of breakage and check, and structural composition of eggs were used to determine egg quality using generally accepted methods. The study [26] reports on the effects of a combination of shungite from the Republic of Karelia's Zazhoginsky deposit and zeolite from the Republic of Tatarstan's Shatrashansky deposit on the indicators of natural resistance, growth, and productive qualities of broiler chickens in combined mycotoxicosis. The experiments were performed in vivarium conditions at the Federal center of toxicological, radiation and biological security. In the experiments, broiler chickens of the meat cross Cobb 500 weighing 0.68-0.73 kg were used. The duration of the experiment was from 21 days of age to the day of control slaughter, which was performed at the age of 42 days. To exclude the influence of feed additives on the birds' organism, birds in the first - control group received complete mixed fodder, while birds in the second group were supplemented with a mixture of shungite and zeolite (the ratio in the mixture 70:30%) in the main diet at the maximum dosage studied - 0.5% of the diet. In the third group, chickens were fed the toxic diet; in the fourth and fifth groups, chickens were fed a mixture of sorbents in two dosages - 0.5 and 0.25% of the diet, respectively, in order to determine the optimal dosage of feed additive use. The toxic diet was prepared on the basis of compound feed for broiler chickens with the addition of mycotoxins: T-2 toxin (200 µg/kg), zearalenone (500 µg/kg) and aflatoxin B1 (50 µg/kg). As a result of the conducted research, it was found that the mixture of shungite and zeolite has high efficiency. Following its use, the organism's natural resistance increases, as does the average daily weight gain. The most cost-effective and optimal dose of 0.5% of the diet should be considered for mycotoxicosis, as a lower dose (0.25% of the diet) is less effective [27].

4. Conclusions

The mineral shungite and brown algae from the genus *Fucus* were chosen as natural adaptogens, according to the research plan. Raw material preparation included milling and classification to fractions with particle sizes ranging from 0.2 to 0.8 mm. The additive samples were tested for toxicity using physicochemical and toxicological methods. It was found that there was no toxicity in the feed additives. All standard toxic elements in shungite and algae feed additives were found to be within MPC. The safety of feed additives and veterinary drugs was determined by biotesting. All samples were safe.Feeding experiments were conducted on young laying hens of the Highsex white cross. The formulation composition was developed to improve chicken immune status, accelerate the development of internal organs in to assume that administering shungite enhances the effectiveness of the immunizations. Addition of fucus crumbles to the diets of young birds did not have a significant effect on the livability of birds compared to the control variant. The findings of the study will be used in poultry farming to improve egg weight and quality, the content of crude protein, carotenoids, and vitamin A in egg mass, the digestibility of amino acids lysine and methionine, hemoglobin content, immunity, and the incidence of fatty deposits in the liver.

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