Anaerobic Fermentation of Chicken Manure and Methods for Intensifying Methane Output

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Abstract. A considerable share in the formation of food security of the population is occupied by the poultry industry, which is one of the most economically attractive and competitive, as evidenced by the annual steady growth dynamics of the production of valuable food products – poultry meat and eggs, characterised by high nutritional value, excellent dietary and taste qualities. The increase in poultry production leads, respectively, to the intensification of production on the one hand, and on the other hand – the accumulation of waste due to an increase in the number of poultry. One of the priority areas for solving the problem of environmental safety in the poultry industry is the processing of animal by-products through enzymatic fermentation, which yields an added energy product and organic-mineral fertiliser, thereby preventing methane emissions into the atmosphere, and therefore global warming. Therefore, the search for ways to intensify the methane output from chicken manure upon anaerobic fermentation, namely by adding various substances, was the purpose of the planned study. The experiment was conducted using laboratory, analytical, and mathematical-statistical methods. According to the results of experimental studies, a positive effect of FeO, Fe₂O₃ was established, Basidiomycota fungal-based bio-compositions, biologics – Meganit Nirbator, Reduklin T, Reduklin Compost and a complex preparation for activating enzymatic processes in chicken manure on the processes of anaerobic bio-fermentation and growth of methane content (CH₄) from chicken manure (in vitro) against the background of an increase in the pH value to 9.05-9.3 with a simultaneous lower level of carbon dioxide (CO₂). Best results for increasing the volume of CH₄ output from the fermented substrate, by 15.7-18.8%, was observed in variants with a complex preparation for activating enzymatic processes in chicken manure. Application of Basidiomycota fungal-based bio-composition contributes to an increase in methane emissions from the test substrate by 5.4-9.6%, and biologics – Meganit Nirbator, Reduklin T and Reduklin Compost cause an increase in the volume of this gas output, respectively, by 5.6-9.4%, 9.5-14.2%, and 7.1-12%. Adding FeO and Fe₂O₃ to chicken manure causes an increase in the level of CH₄ emissions by 4.1-7.4% and 5.8-11.2%, respectively. Thus, the results obtained indicate the expediency of using the studied substances in the processing of chicken manure in biogas plants to intensify the methane yield, which will minimise the adverse impact of intensive management of the poultry industry on the state of the environment.

Keywords: poultry industry, animal by-products, methanogenesis, test substances, greenhouse gases
INTRODUCTION

Agriculture constitutes a priority sector of the Ukrainian economy, which allows obtaining considerable amounts of food, both for saturating the Ukrainian market and for exporting abroad (Zakharchenko, 2107). The products of agricultural enterprises are the key to food security and independence of the country (Zakharchenko, 2107; Selivestrova, 2018). In the overall structure of agriculture, animal husbandry is a strategically important industry, as it accounts for more than 38% of gross output (Selivestrova, 2018; Khodorchuk et al., 2014). The development of animal husbandry, on the one hand, provides the population with high-quality, high-calorie, and dietary food products, and, on the other hand — organic fertilisers, and thus leads to an increase in the anthropogenic load on the environment, and therefore adversely affects the state of public health (Demchuk et al., 2010; Palapa et al., 2016).

One of the most promising branches of animal husbandry is poultry farming, the share of which in the industry structure is quite considerable and accounts for about 40-65% of the total production of livestock products and is also one of the main producers of animal protein necessary for the human body (Fudrycko et al., 2019). Today, poultry farming is the only industry in Ukraine that has been increasing its capacity over the past decade (Selivestrova, 2018; Boroday et al., 2017). However, the growth of the poultry population in poultry farms is accompanied not only by an increase in the volume of the main products — eggs and meat, but also by the development and accumulation of animal by-products in a much larger amount, including chicken manure (Fudrycko et al., 2019; Boroday et al., 2017; Zhukov, 2016). One bird releases 1.1-1.5 times more manure per day compared to the amount of feed it consumes during this period (Skliar et al., 2018). It is known that during the year, 250-300 eggs are obtained from the laying hen, i.e., 15-18 kg of egg mass, which exceeds its weight by more than five times, while during this period the hen releases 40-65 kg of manure with a humidity of 65-75% (Zhukov, 2016). Thus, considering the above, the poultry industry is one of the environmental pollutants, since the use of manure without appropriate preparation as fertilisers adversely affects the ecological state of territories, which carries a direct biological threat due to the presence of pathogens of infectious and especially invasive diseases (Demchuk et al., 2010; Shevtsova & Solohub, 2019; Bolan et al., 2010). As a result, ecosystems in the zone of functioning of poultry farms gradually lose their biological balance and ability to self-regulate, and they experience a sharp deterioration in the growth and development of flora and fauna (Boroday et al., 2017). Forests and fields adjacent to poultry enterprises are beginning to act as distribution centres for pathogenic microorganisms and heavy metals, etc., instead of a buffer function (Shevtsova & Solohub, 2019). Thus, unsatisfactory storage and irrational use of manure not only causes considerable damage to the environment, polluting land reservoirs, soils, and groundwater, but also causes the loss of an enormous amount of high-quality organic fertiliser needed for agricultural land (Demchuk et al., 2010; Shevtsova & Solohub, 2019; Bolan et al., 2010). Long-term accumulation of waste causes intensive fermentation processes, as a result of which gaseous aeropollutants — methane, carbon dioxide, etc. — evaporate into the atmosphere and enter in large quantities (Khodorchuk et al., 2014; Caro, 2019; Rubežius et al., 2020).

The growing concentration of greenhouse gases in the atmosphere increases the greenhouse effect, adversely affects natural processes due to intensive retention of thermal radiation, which contributes to excessive warming of the planet, an increase in the number of natural disasters and cataclysms (desertification, landslides, hurricanes), etc. (Khodorchuk et al., 2014; Vorobel et al., 2021; Monteny et al., 2001). Climate change due to the greenhouse effect is one of the most global environmental problems of modern times (Khodorchuk et al., 2014; Demchuk et al., 2010; Caro, 2019). As a result of the analysis of literature sources, it was found that the potential of methane influence on atmospheric heat retention is 21-34 times stronger than carbon dioxide, and therefore, in conditions of increasing the average annual temperature, CH₄ emission into the atmosphere worsens these conditions (Caro, 2019; Mitkov et al., 2012; Asgedom & Kebreab, 2011). At the same time, methane can remain in the atmosphere for up to 12 years (Mitkov et al., 2012). Hence, reducing the CH₄ output is more effective in preventing climate change than reducing CO₂ emissions. The concentration of methane in the atmosphere has more than doubled over the past two centuries, and carbon dioxide has increased by more than 25% (Binkovska & Shanina, 2016). Thus, in modern conditions, due to the active development of the poultry industry, and therefore a considerable amount of waste accumulation, which, albeit a valuable raw material, is not always used, the urgency of the problem of environmental protection increases. Considering the above, the issue of reducing greenhouse gas emissions from animal by-products is relevant, which requires thorough research and constitutes an essential aspect in the functioning of agro-industrial enterprises.

Anaerobic bio-fermentation of organic animal waste, namely poultry farming is an effective and rational way of their neutralisation, processing, and disposal in modern economic conditions, and therefore the vector of solving problems of eliminating the available and preventing further environmental pollution and ensures waste-free production, i.e., it allows increasing the quantity and improving the quality of organic fertiliser and obtaining an additional energy carrier — biogas, the main component of which is methane (Soluk et al., 2015; Dere et al., 2017; Wang et al., 2019). The use of animal by-products due to the presence of a considerable raw material potential suitable for fermentation in the agricultural sector as alternative energy sources is one of the promising, environmentally friendly and energetically profitable areas of bioenergy today, reducing the amount
of pollutants released into the atmosphere (Panchuk & Shlapak, 2016; Abbasi et al., 2012; Ulusoy et al., 2021). Processing of organic waste from poultry farms by biological fermentation is of great environmental importance, since all chemicals contained in organic waste are completely disposed of (Mitkov et al., 2012). Furthermore, the bio-fermentation process allows destroying the pathogenic microflora and weed seeds, i.e., improves the properties of manure as fertilisers, thereby minimising water, air, and soil pollution (Demchuk et al., 2010; Mitkov et al., 2016; Binkovska & Shanina, 2016).

Methane fermentation is a multi-stage process of microbiological transformation of an organic substrate carried out by a complex consortium of anaerobic bacteria in an oxygen-free environment to final products, mainly methane (55-70%) and carbon dioxide (30-45%) and less than 1% of other gases (hydrogen sulphide, ammonia, aromatic hydrocarbons, etc.) (Dere et al., 2017; Adekunle & Okolie, 2015; Ziemiński & Frąc, 2012). First, during hydrolysis, under the action of microorganisms, high-molecular organic compounds of raw materials (proteins, lipids, polysaccharides) are destroyed to low-molecular fatty acids and alcohols, and then they are oxidised by aceticogenic bacteria to form organic acids (lactic, propionic, acetic, etc.) (Demchuk et al., 2010; Soluk et al., 2015; Panchuk & Shlapak, 2016). Hydrolysis also produces hydrogen and carbon dioxide. The above-mentioned compounds, as well as the organic substrate, serve as further nutrient substrates for the development of methane-forming bacteria (methanogens), which carry out the final stage of fermentation-methane synthesis (Abbasi et al., 2012; Ziemiński & Frąc, 2012; Shtatskyi et al., 2013). It is at this stage that 90% of all methane is synthesised, and 70% of it is formed from acetic acid, and therefore the level of the latter constitutes a factor determining the rate of methane formation (Soluk et al., 2015; Shtatskyi et al., 2013). Notably, upon methane fermentation, up to 83% of the energy of fermented glucose is preserved, and therefore methanogenesis is the most energy-efficient way to transform the energy of organic substances (Shtatskyi et al., 2013).

A promising substrate to produce biogas is chicken manure, due to the increased content of organic matter and the inherent greater ability to biological decay, compared to other animal waste (Dere et al., 2017). Given this, the poultry industry is not only a producer of environmental pollution, but also a potential donor of alternative energy due to the use of organic waste biomass, which allows transforming the manure from harmful to the environment to profitable and useful related products, thereby ensuring high competitiveness and profitability of the industry, turning it into a highly efficient sector of the economy.

Thus, if the reduction and increase in the number of animals is suspended, biogas can become a relatively inexpensive alternative to natural gas, and compared with other renewable energy sources, it is very flexible in use, namely it is used in three important areas – the production of heat, electricity, and fuel. Thus, considering the relevance of ensuring timely and proper disposal of animal by-products in the conditions of intensification of poultry farming, it becomes relevant to develop and search for effective means and methods to activate methanogenesis, which will speed up the fermentation processes of waste, thereby contributing to the growth of production of renewable energy resources, obtaining valuable humus, and ensuring the main component of value-preservation and protection of the environment, and therefore minimise the consequences of global warming.

The purpose of this study was to investigate the anaerobic fermentation of chicken manure (in vitro) and establish the effectiveness of the influence of the substances under study on the intensification of methane output, which directly determines the value of biogas.

MATERIALS AND METHODS

Experimental studies on the effectiveness of the influence of the substances under study on the emission of greenhouse gases — methane and carbon dioxide from chicken manure, were carried out using laboratory methods — to determine the level of greenhouse gas release; analytical methods — to analyse and justify the results obtained; mathematical and statistical methods — to assess the reliability of research results. The study is based on the methodology of O.H. Skliar, R.V. Skliar, and S.M. Hryhorenko (2019). Sampling of the substrate under study, the chicken manure without bedding, was carried out in the farm “Zakhid-Plytsia” of the Lviv Oblast. The methane fermentation process was carried out in vitro using sealed containers to maintain hermeticity and ensure anaerobic conditions. To maintain the stability of the bio-fermentation process and avoid its inhibition by ammonium nitrogen and sulphides, biomass (chicken manure) was diluted with water to a humidity of 92%, since it is known that for the effective anaerobic fermentation, and therefore an increase in methane yield, high humidity substrates are necessary. During the experiment, the fermented mass was mixed by periodically shaking the containers to destroy and prevent the formation of a crust, which leads to a delay in the release of gases. The bio-fermentation conditions were identical, both in the control version, where anaerobic digestion of the substrate was carried out at the expense of the natural microflora of the manure, and in experimental analogues with the introduction of the substances under study.

Experimental studies were carried out within 26 days, and after the stages of hydrolysis, oxidation, and acetogenesis at the beginning of methane formation (day 17), the substances under study were added to fermented chicken manure — methane generating raw materials in an effective pre-established and economically justified optimal dose of 3%: Variant I — control (without adding substances); II — FeO, 9 g; III — Fe₂O₃, 9 g; IV — Basidionymycota fungal-based bio-composition (Institute of Agroecology and Nature Management), 9 ml; V — biological product Meganit Nirbator (PE “Eksiminvest”), 9 ml; VI — biological product Reduklin T (PE “Agro-Admiral”), 9 ml; VII — biological product
Reduklin Compost (PE “Agro-Admiral”), 9 ml; VIII – complex preparation for activating enzymatic processes in chicken manure (Institute of Agriculture of the Carpathian region), 9 ml. During the experiment in the in vitro conditions, on the 17th day and every three days, the level of greenhouse gas emissions from the substrate under study (CH$_4$, CO$_2$) was determined in control and in experimental analogues. The released amount of the gases under study upon anaerobic fermentation of chicken manure was measured using a portable gas analyser — Dozor S-M-5 (Certificate of verification of the device type UA.TR.001 212-18 and certificate of conformity UA.TR.002.CB.1234-19). In the experiment, the acidity of the test substrate was also determined (at the beginning of experimental studies and after completion) using the Tur ns170 pH meter device.

Statistical analysis of the obtained research results was carried out using a standard package of statistical software, Microsoft EXCEL and AtteStat using variational statistics methods. Arithmetic mean values (M) and their errors (m) were calculated. The difference between the arithmetic mean values was considered statistically significant at: *P<0.05; **p<0.01; ***p<0.001.

RESULTS AND DISCUSSION

To ensure maximum efficiency of the anaerobic fermentation process, i.e., active reproduction and enzymatic activity of microorganisms, it is necessary to maintain optimal conditions, namely the temperature regime and acidity (pH) of the medium, etc. (Dere et al., 2017; Adekunle & Okolie, 2015; Ziemiński & Frąc, 2012). According to several studies, it has been established that the amount of methane produced during anaerobic digestion is largely determined by temperature (Dere et al., 2017; Ziemiński & Frąc, 2012; Polishchuk et al., 2013). In particular, the minimum temperature at which methanogenesis occurs is 6ºC, while its value below 0ºC is maintained by microorganisms, but the metabolic process completely stops (Vorobel et al., 2021; Soluk et al., 2015; Ziemiński & Frąc, 2012). An increase in temperature contributes to a higher rate and degree of destruction of organic raw materials, but at a level of more than 70ºC, methane-forming microorganisms die, and at a value above 45ºC, the methane concentration in the total volume of gases decreases, despite the improvement in the conditions of their development (Skliar et al., 2018; Soluk et al., 2015; Polishchuk et al., 2013). The above can be explained by the fact that at elevated temperatures, carbon dioxide dissolved in the substrate is more intensively converted to a gaseous state and released, and the more of it passes into a gaseous form, the lower the proportion of methane will be (Polishchuk et al., 2013). In addition, anaerobic fermentation at elevated temperature increases the sensitivity of microorganisms to its permissible fluctuations, which causes a decrease in their metabolic activity and ability to reproduce, so sharp changes in this indicator are not desirable (Ziemiński & Frąc, 2012; Polishchuk et al., 2013). Considering the above, experimental studies were carried out at a temperature within 33ºC, i.e., under the mesophilic regime, which is described by the highest stability of anaerobic digestion and minor temperature fluctuations are allowed without disrupting the process. The duration of substrate bio-fermentation is also one of the key indicators affecting the properties of manure, their digestibility, reduction of toxic substances and harmful microorganisms, etc. (Skliar et al., 2018; Soluk et al., 2015). In particular, the fermentation exposure is set depending on the temperature regime within the following limits: at 10-25ºC for 30 days, at 25-40ºC — for 10-20 days, at 45-55ºC — for 4-8 days (Adekunle & Okolie, 2015; Polishchuk et al., 2013). In this experiment, the duration of the substrate bio-fermentation process was 17 days.

The stability of methanogenesis is considerably influenced by the hydrogen index, which determines the vector of anaerobic fermentation (Dere et al., 2017; Ziemiński & Frąc, 2012; Polishchuk et al., 2013). According to literature sources, it is known that a low pH level inhibits the growth of methanogenic bacteria and reduces the methane yield, specifically when this indicator decreases below 6.5, the gas yield worsens by 30-40%, and the development of methane microflora almost completely stops at pH 6.0 (Polishchuk et al., 2013). In this experiment, upon determining the pH in the control variant (without adding substances) of chicken manure before and after the study (in vitro) was completed it was found that this indicator was within 8.35-8.55, i.e., it had an alkaline reaction of the medium. During experimental studies, there was a change in the acidity index. During the period of the bio-fermentation process in the experimental variants, after the introduction of the substances under study into the substrate, an increase in the enzymatic activity of the fermented mass was observed, and after the studies were completed, the pH level increased to 9.05 in variants using FeO, to 9.1 — using Fe$_3$O$_4$, to 9.2 — using Basidiomycota fungal-based bio-compositions, to 9.15 — using Meganit Nibator biologics, to 9.28 — using Reduklin T biologics, to 9.25 — using Reduklin Compost biologics, to 9.3 — using a complex preparation for activating enzymatic processes in chicken manure (Fig. 1).
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According to the results of the conducted studies, it was established that upon anaerobic fermentation of chicken manure (in vitro) in variants using FeO, the methane yield from the test substrate increases, depending on the day of the experiment, namely: on day 20-22 l/m³ (P<0.01) or 4.1%; on day 23-31 l/m³-5.7%; on day 26-41 l/m³, i.e., 7.4%, relative to the control analogue. Introduction of Fe₂O₃ to fermented chicken manure causes an increase in greenhouse gas emissions, such as methane, relative to the control variant, depending on the day of research, namely: by 31 l/m³ (P<0.001), i.e., 5.8% — on day 20; by 46 l/m³ (P<0.01) or 8.5% — on day 23; by 62 l/m³ (P<0.01) or 11.2% — on day 26.

Therefore, maintaining optimal parameters of methane fermentation is a substantial factor determining the yield of biogas, the speed of the process and the methane content, and ultimately the efficiency of processing organic raw materials. At the same time, a promising area for intensifying the methane fermentation process is the use of stimulants that help improve the vital activity of microorganisms that carry out transformation processes. The analysis of the obtained results shows that the substances under study, which contributed to the highest pH indicator in the fermented chicken manure, and therefore to the acceleration of bio-fermentation processes, simultaneously showed the most effective influence on the methane output increase (Fig. 2).

The data obtained from experimental studies indicate that the intensity of the anaerobic fermentation process and the emission of greenhouse gases — methane and carbon dioxide from the substrate of chicken manure is determined by the duration of the experiment. With increasing fermentation time, the methane content increases, which indicates an improvement in the biogas quality.

According to the results of the conducted studies, it was established that upon anaerobic fermentation of chicken manure (in vitro) in variants using FeO, the methane content increases, depending on the day of the experiment, namely: on day 20-22 l/m³ (P<0.01) or 4.1%; on day 23-31 l/m³-5.7%; on day 26-41 l/m³, i.e., 7.4%, relative to the control analogue. Introduction of Fe₂O₃ to fermented chicken manure causes an increase in greenhouse gas emissions, such as methane, relative to the control variant, depending on the day of research, namely: by 31 l/m³ (P<0.001), i.e., 5.8% — on day 20; by 46 l/m³ (P<0.01) or 8.5% — on day 23; by 62 l/m³ (P<0.01) or 11.2% — on day 26.

**Figure 1.** The level of acidity of chicken manure in variants using the substances under study

**Figure 2.** Volume of methane output from chicken manure in variants using the substances under study
Analysis of the data obtained during this study shows that the addition of the Basidiomycota fungal-based bio-composition to the substrate under study upon anaerobic fermentation (in vitro) promotes an increase in the volume of CH\textsubscript{4} output for 20-26 days, respectively, by 29-53 l/m\textsuperscript{3} (P<0.05), which is 5.4-9.6% as a percentage compared to the control variant.

According to the results of studies, it was found that upon using the biological product Meganit Nibtator, there is an increase in the level of methane emissions from fermented chicken manure, relative to the control analogue, depending on the day of research, respectively: on day 20-30 l/m\textsuperscript{3} (P<0.05), i.e., 5.6%; on day 25-45 l/m\textsuperscript{3} (P<0.05) or 8.3%; on day 26-52 l/m\textsuperscript{3} (P<0.01), which is 9.4%.

In variants with the introduction of Reduklin T into the test substrate, the volume of CH\textsubscript{4} output increases depending on the day of the experiment, namely: by 51 l/m\textsuperscript{3} (P<0.05) or 9.5% — on day 20; by 70 l/m\textsuperscript{3} (P<0.05) or 12.8% — on day 23; by 78 l/m\textsuperscript{3} (P<0.05) or 14.2% — on day 26 of the experiment, compared to the control variant. Based on the conducted studies, it was found that adding the biological product Reduklin Compost to fermented chicken manure upon anaerobic fermentation (in vitro) causes an increase in methane emissions by 20-26 days, respectively, by 38-66 l/m\textsuperscript{3} (P<0.05-0.01), i.e., 7.1-12%, relative to the control variant.

During the same period of experimental studies (20-26 days), the volume of CH\textsubscript{4} output from the test substrate upon using a complex preparation to activate enzymatic processes in chicken manure, it was higher simultaneously with an increase in methane levels upon anaerobic fermentation (20-26 days), respectively, by 20-26 days, (P<0.05-0.01), i.e., 15.7-18.8%.

Thus, considering the obtained research results, the highest level of methane released from fermented chicken manure in all experimental versions is observed on the 26th day of the experiment — by 7.4-18.8%. At the same time, the most pronounced effect (15.7-18.8%) on the growth of the CH\textsubscript{4} output from the test substrate, a complex preparation for activating enzymatic processes in chicken manure was developed during the experiment. The intensification of the process of bio-fermentation of chicken manure and the highest level of methane in the experimental variants under study is probably conditioned upon the wide component composition of bio-stimulants that provide methanogenic microorganisms with a sufficient amount of required nutrients, including mineral elements (Demirel & Scherer, 2011). The above is confirmed by the studies of several researchers on the crucial role of mineral elements (Fe, Ni, Co, Mo, W, and Se), enzymes, microorganisms in the growth and metabolism of methanogens and for further acceleration of methane biosynthesis, as well as improving the stability of anaerobic fermentation (Zhang et al., 2013; Christy et al., 2014; Romero-Gująza et al., 2016). A mineral element such as Fe increases the production of acetate and at the same time can directly serve as an electron donor for the reduction of CO\textsubscript{2} to CH\textsubscript{4} (Romero-Gューza et al., 2016). The results obtained during the experiment on increasing the efficiency of methanogenesis, namely the formation of methane from the fermented substrate, are consistent with the studies of E. Abdelsalam et al. (2015), conducted in laboratory conditions using chlorine compounds CoCl\textsubscript{2}, NiCl\textsubscript{2}, and FeCl\textsubscript{3}. Researchers J. Ahamed et al. (2016), R. Zeng et al. (2021) and J. Pan et al. (2019) obtained comparable results regarding the stabilisation of the anaerobic process, and consequently an increase in the yield of biogas with a high methane content from poultry industry waste using silica gel and biochar.

Analysis of experimental data shows that simultaneously with an increase in methane levels upon anaerobic fermentation of chicken manure (in vitro) in all experimental versions, carbon dioxide emissions are reduced (Fig. 3). When Fe compounds are added to the test substrate in the forms of FeO and Fe\textsubscript{3}O\textsubscript{4}, for 20-26 days, there is a decrease in the volume of CO\textsubscript{2}, respectively, by 3-9 l/m\textsuperscript{3}, i.e., 0.8-2.3%.

![Figure 3. Volume of carbon dioxide output from chicken manure in variants using the substances under study](Image)
Introduction of Basidiomycota fungal-based bio-compositions to fermented chicken manure according to the mesophilic regime of anaerobic fermentation (in vitro) helps reduce the carbon dioxide release, depending on the day, respectively, by 19 l/m³ (P<0.01) or 4.9% — on day 20; by 32 l/m³ or 8.2% — on day 23; by 38 l/m³ (P<0.01) or 9.6% — on day 26, relative to the control variant.

Based on the conducted studies, it was found that the use of Meganit Nirbator biologics causes a decrease in CO₂, yield from the test substrate, respectively, on day 20-20 l/m³ (P<0.01) or 5.1%; on day 23-50 l/m³ (P<0.01) or 7.7%; on day 26-37 l/m³ (P<0.01) or 9.4%, relative to the control variant.

Analysis of the results obtained shows that upon adding the Reduklin T biological product, there is a decrease in carbon dioxide emissions from fermented chicken manure, depending on the day of the experiment, respectively, by 33 l/m³ (P<0.05) or 8.5% — on day 20; by 42 l/m³ (P<0.01) or 10.8% — on day 23; by 49 l/m³ (P<0.01) or 12.4% — on day 26, compared to the control variant.

In variants with the introduction of the biological product Reduklin Compost for the study period (20-26 days), the yield of CO₂ decreases from the test substrate, respectively, by 25-39 l/m³ (P<0.05-0.01) or 6.4-9.9%, relative to the control variant.

According to experimental data, it was found that upon using a complex preparation to activate enzymatic processes in chicken manure, the volume of carbon dioxide output from the fermented substrate, depending on the day of the experiment, is lower, respectively, on day 20-50 l/m³ (P<0.01) or 12.9%; on day 23-53 l/m³ (P<0.01) or 13.6%; on day 26-56 l/m³ (P<0.01) or 14.2%. Thus, analysing the research results, on day 26 of the experiment, the lowest level of CO₂ emission was established in all experimental variants upon mesophilic bio-fermentation (by 2.1-1.4%) from chicken manure. In particular, the most effective reduction of carbon dioxide output from the fermented substrate was shown by a complex preparation for activating enzymatic processes in chicken manure, namely — by 12.9-14.2%, depending on the day of the experiment (20-26 days). Considering the above, it is experimentally confirmed and economically justified that the test substances FeO, Fe₂O₃, Basidiomycota fungal-based bio-composition, biologics — Meganit Nirbator, Reduklin T, Reduklin Compost and a complex preparation for activating enzymatic processes in chicken manure effectively influence the growth of CH₄, yield volume from a fermented substrate in the mesophilic mode of anaerobic fermentation (in vitro) with a simultaneous decrease in CO₂ release.

Thus, of the substances under study, the most effective in increasing methane emissions at a lower level of carbon dioxide from the fermented substrate was found in variants using a complex preparation to activate enzymatic processes in chicken manure and, accordingly, their positive effect on the process of methanogenesis decreases in the following sequence: Reduklin T — Reduklin Compost — Fe₂O₃ — Basidiomycota fungal-based bio-composition — Meganit Nirbator — FeO.

CONCLUSIONS
The effectiveness of the substances under study, namely FeO, Fe₂O₃, Basidiomycota fungal-based bio-compositions, biologics — Meganit Nirbator, Reduklin T, Reduklin Compost and a complex preparation for activating enzymatic processes in chicken manure to intensify the bio-fermentation and methane release from chicken manure (in vitro) is theoretically justified, which is conditioned upon changes in the pH of the substrate to the alkaline side, while reducing the level of carbon dioxide emissions. It has been experimentally confirmed that the most influential on methanogenesis, and therefore the growth of CH₄, yield from chicken manure developed is a complex preparation for activating enzymatic processes in chicken manure (15.7-18.8%), biologics — Reduklin T (9.5-14.2%) and Reduklin Compost (7.1-12%). Thus, the increase in the level of methane upon the use of the substances under study indicates the prospects of their application in the processing of chicken manure in biogas plants for a comprehensive solution simultaneously with the energy and environmental problem, i.e., to reduce environmental pollution with intensive management of the poultry industry, which allows improving the profitability of both husbandry and crop production by obtaining high-quality humus suitable for organic production.

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