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**ASSESSMENT OF THE ENVIRONMENTAL RISK DUE TO MODIFICATION IN THE NITROGEN CIRCUMROTATION ECO-TECHNICAL SYSTEM FOR EGGS**

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**Adelina Gencheva**Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria, [adelina.g@abv.bg](mailto:adelina.g@abv.bg)**Veselin Kirov**University of Forestry Faculty of Veterinary Medicine, Sofia, Bulgaria, [kirovvk@gmail.com](mailto:kirovvk@gmail.com)

**Abstract:** At the end of the 19<sup>th</sup> century Clarke (evidenced by Dobrovolskiy, 1990) finds that the lithosphere is chemically inhomogeneous, and Vernadsky (1923) proves that the chemical inhomogeneity is typical for the entire biosphere. When studying the eco-technical systems for eggs and meat the “Clarke of concentration” criterion has been applied to describe the movement of toxic elements in the following unit of the trophic chain: bioproducers /fodder + water/ - primary bioconsumers /poultry and their production/ /Baykov, B.,1987, Baykov & Tyrawska, 1991, Baykov, B.,1994. Baykov et al., 1996/

Trials are carried out in an integrated two-module model of an eco-technical system for egg production. The two main types of trophic chains are modelled: pasture type and detritus type. Insufficiently effective use of nitric compounds from the ration for synthesis of specific egg whites is found.

Concentration of total nitrogen in the manure, as well as high content of non-protein nitric, nitrite and ammonium nitrogen is proved in comparison with the amounts at the system inlet.

The consequences of this distribution in the two products of the outflow /egg mass and manure/ for the human health and ecosystems are studied. The content of toxic non-protein compounds of nitrogen in the egg mass used as human food resource is below the admissible values in accordance with the EC Regulation on foods, and therefore the assessment shows that there is no direct risk for the human health.

The indirect ecological risk related to the consequences from the linearity of technologies is significant: obtaining of manure with high content of nitric compounds, which are changed for all the three modelled technologies for manure use by increasing the non-protein nitrogen. Significant nitrogen losses are found in the two trial aerobic technologies for manure processing.

It is most ecologically feasible to process manure in industrial biogas installation that ensures conditions for intensive and controlled anaerobic decomposition, where biogenic elements are fully preserved, where no greenhouse gas emissions are observed, and where, in addition to biosludge, which is an alternative of the highly energy consuming mineral manures, gas fuel is generated /biogas/.

**Keywords:** eco-technical system, eggs, organic manure, anaerobic decomposition, composting, manure aging.

## 1. INTRODUCTION

The studies on chemical heterogeneity in the eco-technical systems have continued with regard to the chemical heterogeneity at organism level and the “Clarke of safety” criterion has been proposed for the assessment of the food safety-related environmental risk /Kirov et al., 2018/. Our concept for ecologisation of technologies in stock-farming comprises the requirement when managing the eco-technical system to analyse and regulate not only the circumrotation of elements in the trophic chain but also all biogenic elements of lithospheric origin, among which nitrogen is of primary importance for the heterotrophic organisms. Gencheva /2015/ suggests to use the term “retention” to express in quantitative aspect the degree of transformation of nitric compounds in the ration within the egg whites, i.e. in the secondary biological production of phytophages /laying hens/ used as human food. These studies contribute to the expansion of researches on the chemical heterogeneity of nitrogen in an eco-technical system for eggs emphasizing on the environmental risk caused by the nitric compounds to the human health and the ecosystems.

## 2. MATERIAL AND METHODS

Trials have been carried out in an integrated model of eco-technological system for eggs, at “mesocosm” level as per Odum’s classification /1986/, which comprises two modules. In the first module a trophic chain of pasture type is modified, which is characterized with broken circumrotation of the matter and input of large amounts of nitric compounds from ecotops that are internal for the system. The second module – a model of detritus type, is presented in three versions, which reproduce technologies applied in the practice: biotechnological chain for anaerobic decomposition of manure’s organic substances /generation of biogas and biosludge/, for aerobic decomposition

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/compositing generation/, and model for manure aging and extensive mineralization that reproduces the best farming practices.

The first module /a trophic chain of pasture type/ comprises anthropogenically modified ecotop: air-conditioned facilities in a trial farm.

Abiotic parameters of the living environment are in compliance with Ordinance № 44 of the Ministry of Agriculture and Food, and namely: air temperature 16-22°C, relative humidity 50-72%; air movement velocity within the living area -0,2 -0,5 m/sec., light intensity – 40 lux, toxic gases content – carbon dioxide - 0,1%, ammonia and hydrogen sulphide – traces. Poultry are being watered from a central water source, whereas the water meets the requirements of Ordinance № 9/2001 of the Ministry of Health. A group of 80 poultry is introduced with the ecotop and the animals are at the same age, with the same biomass and health status, of the Gallus gallus type, laying hens, breed (ISA-Brown) at the age of 18 weeks, which are being bred for a period of 300 days.

During the trial period, poultry receive fodder with the following content: 18% raw protein, 0,44% digestible phosphorus, 3,8 3% calcium, 0,91% lysine, 0,76%, methionine + cysteine and metabolic energy 2750 kcal/kg fodder. Poultry are being fed and watered without restrictions.

Biotic factors are regulated by means of applying the prophylaxis programmes that are well-established in practice and by means of controlling the abiotic factors /intensity of air exchange/ in order to limit the microbial contamination of the air. Poultry are being bred as floor poultry, without litter, with density of 6 hens/m<sup>2</sup> of floor area.

The second module /biotechnological chain for biogas generation/ comprises modelling of anaerobic decomposition /AD/ of biomass in a laboratory fermenter of cascade type, developed by us, with ability to regulate the technological parameters in accordance with the results of the mathematical modelling of the process of anaerobic decomposition of biomass. For the purposes of optimizing the methane fermentation, we applied the model of Chen and Hashimoto /1979/, which has been used by a number of authors working with laboratory, pilot and industrial biogas installations. It has been applied to a wide range of values of different variables and to different substrates (Chen & Varel, 1980, modification described by Baykov & Tyrawska, 1991/

The second version of a biotechnological system is a model of controlled aerobic mineralization realised in laboratory installation for composting.

The third module reproduces aerobic decomposition of manure's organic substances under extensive, non-controlled conditions, widely applied in practice and known as "Good Agricultural Practices". For the needs of the trial we used a large-scale model of manure depot made of resistant plastics, with storage duration of 180 days.

Laboratory tests are carried out in accordance with the following algorithm:

Eggs are collected and tested on daily basis. The white, the yolk and the shell are separated and weighed separately. Samples are stored at 0°C until the time of testing /every 10 days/.

Samples are being taken on daily basis from manure /fresh and aging/, biosludge and compost and regularly – every 10 days, from fodder and potable water. These samples are tested in accordance with the following standard methods:

- Determination of the mass in fresh condition /by means of weighing/
- Determination of the response of the environment /pH/— BDS EN 12176: 2000
- Determination of dry residue and water content to BDS EN 12880: 2003.
- Determination of total organic Carbon — EN 13137: 2005.
- Determination of general Nitrogen — BDS 11261 ISO: 2002
- Determination of mineral Nitrogen forms: ammonium /NH<sub>4</sub><sup>+</sup>-N/ and nitrate /NO<sub>3</sub><sup>-</sup>-N/ Nitrogen — BDS ISO 14255: 2002.

Statistical analyses. The statistical analysis of data was done using Microsoft Excel (Microsoft Office 2000) and STATISTICA.

### 3. RESULTS

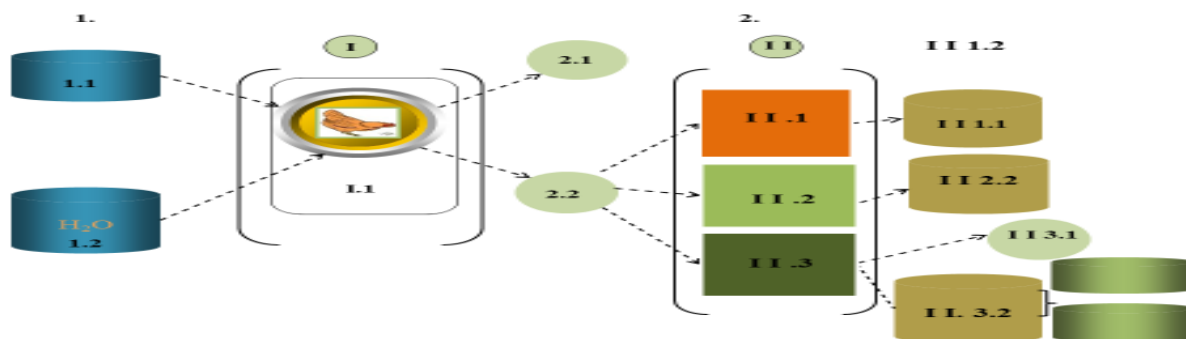
Table 1 and figure 1 present a summary of the results from the nitrogen movement in the integrated model of eco-technical system comprising the two modules that reproduce the two types of trophic chains: of pasture and detritus type.

Table 1. Movement of nitrogen in an eco-technical system for eggs

№	Indication	Unit	Quantity of total nitrogen	Non-protein nitrogen nitrates	Non-protein nitrogen nitrites	Non-protein nitrogen – ammonium
1	Trophic chain of pasture type					
1.1	Combined fodder	mg/kg	28800+42.15	242.10+4.11	-	-
1.2	Drinking water	mg/l	44.18+2.16	44.18+2.16	0.10+0.05	-
1.3	Egg – yolk	mg/kg	47.72+0.82	110.21+4.11	-	-
1.4	Egg – white	mg/kg	131.70+1.14	94.14+ 2.86	-	-
1.5	Egg-shell	mg/kg	9.20+0.42	42.16+3.14	-	-
1.6	Fresh manure	mg/kg	7293.09+38.90	288.80+14.20	59.10+9.17	4.86
2	Trophic chain of detritus type					
2.1	Fresh manure	mg/kg	7293.09+38.90	288.80+14.20	59.10+9.17	4.86
2.2	Stale manure	mg/kg	5422.20+41.90	1313.21+21.14	62.18+4.86	4.11+0.86
2.3	Compost	mg/kg	5851.90+30.90	2116.89+42.86	50.11+4.12	22.14+1.86
2.4	Biosludge total	mg/kg	10324.10+48.10	4592.11+44.18	67.12+5.18	20.14+1.87
2.5	Biosludge liquid fraction	mg/kg	8981.90+56.90	3107.43+41.86	88.84+2.14	4.11+1.11
2.6	Biosludge solid fraction	mg/kg	1342.10+28.20	135.54+12.44	18.24+1.86	15.86+2.14

The chemical heterogeneity at ecosystem level /eco-technical system for eggs/ is expressed with significant differences in the transformation of the total nitrogen from the ration in the two main flows of the secondary biological production. The main amount of nitrogen is concentrated in the manure, which contains 25.33% of the input amount of nitrogen, while in eggs the retention of nitrogen is 0,17% in the yolk, 0,45% in the white and 0.03% in the shell, respectively. The chemical heterogeneity with regard to nitrogen is most expressed in the main product of the secondary biological production, which is a human food resource – the eggs, where the difference in the content of this macroelement is of 0.92 mg/100 g in the egg-shell and to 13.17 mg/100 g in the white.

Figure 1. Scheme of movement of total nitrogen in the eco-technical system for egg production /original/. I. Pastoral type trophic chain model; Incoming stream of matter: 1.1. Feed; 1.2. Drinking water; Output -2.1 - Eggs; 2.2. Manure; II. Model of a detritus type Trophic Chain - II.1 - Aerobic Extensive Processing / Good Agricultural Practices / - Stale manure / II.1.1 /; II.2 - aerobic controlled treatment - composting - compost product // II.2.2 /; II.3 - Anaerobic treatment in a biogas plant / II.3 / - products - biogas / II.3.1 /; biosilm // II.3.2 / - of which liquid and solid fraction;



When interpreting the obtained results, we should take into account that the modelled trophic chain that reproduces a farm for intensive egg production is of pasture type, with anthropogenic modification with regard to main technological factors: anthropogenically modified ecotop, which is not a source of resources without bioreducer unit, ultrahigh density of economically useful population, continuous production cycle. In order to achieve the economic indicators preset by the applied technology /high production capacity, low mortality, acceptable cost, etc./ a new technological module is formed, which does not exist in the natural trophic chains of pasture type – production structure, where the multi-component ration comprising ingredients from different regions of the country and the world, with different chemical composition in comparison to the autotrophic organisms of the region, that is necessary for poultry feeding is being prepared. In the model described at the bioproducer level a ration is formed, which is enriched with nitrogen-rich components, thus obtaining nitrogen content of 28800 mg total nitrogen + 242.10 mg non-protein nitrogen /1000 g of the ration. Poultry exhaust about 130g/ day of this protein-rich fodder enriched with energy agents and vitamins, which is equal to about 3800 mg nitric products. In natural ecosystems the nitrogen content in fodder plants is between 800 and 1800 mg/100 g. This means that in case of administering the same amount of food, the organism would receive significantly less nitric compounds.

The day ration further comprises 320 ml of water, which imports 14.14 mg of nitrate nitrogen. This source of nitrogen represents 0.23% of the total amount.

When analyzing the movement of nitrogen along the eco-technological chain for egg production, Gencheva /2015/ suggests the quantitative criterion '*retention coefficient*' (Cr), which like the bioaccumulation criteria – Clarke of concentration, Clarke of safety, etc., allows to make a quantitative assessment of the total nitrogen retention along the trophic chain. It is determined as a percentage ratio between the nitrogen content in the manure or eggs to its content at the level of bioproducers (fodder) x 100 (to be calculated in percentages).

The main amount of nitrogen is concentrated in the manure, which contains 25.33% of the input amount of nitrogen, while in eggs the retention of nitrogen is 0,17% in the yolk, 0,45% in the white and 0.03% in the shell, respectively. We can see the chemical heterogeneity with regard to nitrogen in the main product of the secondary biological production, which is a human food resource – the eggs, where the difference in the content of this macroelement is of 0.92 mg/100 g in the egg-shell and to 13.17 mg/100 g in the white.

Based on the obtained results, we made the conclusion that the enrichment of ration with nitric compounds /amino acids and proteins/, irrespective of the fact that one of the factors determining the high production capacity with view of biogeochemistry is inexpedient, since no amount of nitrogen adequate to the input amount is found in the secondary biological production used as human food. It is obvious that nitric compounds from the ration take part in the catabolic processes mainly as energy agent after preamination and deamination of amino acids.

Obtained results give grounds to recommend a new approach for balancing the ration of laying hens by enriching it with essential amino acids or fodder components that contain them, which are transformed in specific egg whites in the poultry body. Another option is to study biochemical mechanisms for increase of Cr by applying vitamins /for the time being, there is information about the effect of the group B vitamins/ as well as growth stimulators.

Obtained results show that the minimization or exclusion of the environmental risk is a priority for the management of the eco-technical systems.

For the purposes of assessing the environmental risk, we propose complex criteria at two risk levels:

Direct hazards for human health caused by the nitric compounds contained in the eggs

Indirect hazards for human health and ecosystems as a result of remote consequences from importing the formed nitric acids in the biocenoses.

Table 1 shows that the risk of chemical heterogeneity of nitrogen in the eco-technical system for eggs is related with the formation of non-protein compounds of nitrogen, which have different toxicity for the heterotrophic organisms, including the man. The ammonium, nitrite and nitrate form are subject to control. Of all three types of toxic compounds /columns 5, 6 and 7 of table 1/ only nitrates are found in the egg mass. The results in table 1 are assessed in section 1.3 and section 1.4 in accordance with Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. In accordance with the legal regulations, the lowest admissible content of nitrates is up to 200 mg/kg for children foodstuff. Our trials find amounts of nitrates in the egg mass, which are significantly lower than the minimum amounts admissible under the EC Regulation. No nitrites and ammonium compounds are found. These results show that there is no risk of toxic or metatoxic effect to human health as a result of the existence of non-protein forms of nitrogen in the eggs.

For the purposes of assessing the indirect consequences of the import of nitric compounds in the eco-technical system for eggs, we monitored their movement along the entire eco-technological chain, which is provisionally divided into two modules: pasture type and detritus type.

For the purposes of assessing the efficiency of the eco-technological system's functioning, we applied the criterion "Clarke of concentration" /Cc/ we have developed /Baykov, 1987/.

The model shows that 2.721 kg of fodder with content of 78365.8 mg of total nitrogen, and 4.136 kg of water containing 182.8 mg of nitrogen /0.23% of the total amount/ are imported in the eco-technical system for the production of 1 kg of fresh egg mass /white + yolk/.

We obtain food resource – egg mass, with content of 45.34 mg of nitrogen per 1 kg of fresh mass.

Cc for the egg mass is 0.0006, and for the manure – 0.023, which is an indicator for significant dispersion of input amounts /through the ration or the water/ of nitric compounds per unit of native mass of the two main products of the secondary biological production. Nevertheless, we make the conclusion that the input amount of nitrogen is concentrated mainly in the manure. The manure generated by 1 hen for 1 production period of 300 days /18.210 kg/ concentrates 32352.15 mg of nitrogen /in fresh mass/. The manure generated from 1 hen only for 300 days contains 1281.12 mg nitrate nitrogen, 262.17 mg nitrite nitrogen and 21.56 mg ammonium nitrogen.

The criterion Cc allows us to precisely assess the environmental risk caused by the distribution of the nitric compounds in the secondary biological production, respectively, to minimize the environmental risk of importing nitrogen in soil or water basins. If as a result of the trial /through the described model/ we know the amount of nitrogen for production of a unit of egg mass and the Cc of nitrogen in the manure, we can determine the amount of nitric compounds that are released in the manure during the production of the egg mass preset in the technology and realised as goods. The managerial decisions described in this section should be made.

The analysis and making adequate managerial decisions with regard to the formation and functioning of the eco-technological chains for processing of the main product of the linearity of the technological processes – the manure, are an important element in the systematic approach for management of eco-technical systems for eggs. Disturbing consequences for the nature health are found as a result of the currently applied technologies for manure use. The most popular technological solution is the storage of manure for a period of 180 days known as "maturing" or "aging", where in accordance with the "Good Agricultural Practices" applied in Bulgaria results in decontamination and partial mineralization of the manure. When storing the manure for a period of 180 days, nitrogen losses are 25,6%. As a number of studies show /Baykov, 1987, Baykov et al., 2005/, these losses are due to the release of ammonia (about 2/3 of the nitric emissions) and nitric oxides in the atmosphere as a result of the vital function of the microbial cenosis in the manure. Nitric oxides are greenhouse gases with higher and higher greenhouse effect of the carbon dioxide (CO<sub>2</sub>) and the methane (CH<sub>4</sub>).

Due to the redistribution of the chemical elements in the compost, nitrogen losses are relatively low (19,76%) irrespective of the determined release of ammonia and nitric oxides for this technology for aerobic mineralization of the manure. In this case, the decrease of nitrogen due to the release of nitrogen-containing gases is compensated by the redistribution of chemical elements, and mainly by the significant carbon reduction, which is indicated by the mineralization of the substrate of about 40-60%.

The redistribution of chemical elements in the substrate during the biogas generation is due to the release of significant amounts of carbon, hydrogen and oxygen with the biogas (mixture of CH<sub>4</sub> + CO<sub>2</sub>). The process runs in pressurized environment and therefore the amount of nitrogen in the biosludge is increased, respectively, thus eliminating the emissions, however the content of total nitrogen in the biosludge is 42.6% higher than in the fresh manure.

The distribution of nitric compounds during the separation of biosludge, which dry content is about 4% in our trials, is of great practical importance. The liquid fraction contains 87% of the nitric compounds, and the solid fraction - 13%. This distribution gives grounds to recommend the use the solid fraction as fuel /after drying and pelleting/, as it is an insignificant source of nitrogen and other biogenic elements. The availability of large amount of nitric compounds in the liquid fraction is an indicator of the real risk of pollution of surface and underground water as a result of the infiltration of liquids in the vicinity of stock farms or the direct release of the liquid fraction in the water recipients.

The dynamics of the water soluble nitrogen fractions /columns 5, 6 and 7 of table 1/ is of great importance for the agroenvironmental assessment of the trail technologies. Namely these are accessible for the plants, however we should emphasise the importance of organic compounds in the category of total nitrogen /column 4 of table 1/. Therefore, the content of biogenic elements is a key indicator, and the water soluble forms should be analysed as a criterion for the efficiency of the modelling of the bioreducers' unit /notwithstanding the factors of the environment, and in particular, the availability or lack of oxygen during the mineralization of organic substrates/. We need to dose precisely when importing them in the soil while knowing the physicochemical indicators. Soil fertility and structure are determined by two main groups of products: biogenic elements in mineralized form and biogenic elements as organic compounds.



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Irrespective of the nitrogen form, the concentration of nitric compounds in the manure is a real risk for the soils, surface and underground water. For the purposes of limiting the pollution of arable lands caused by stock-farming, the EU Nitrates Directive has been developed and adopted, which is harmonized in the Bulgarian legislation with Ordinance № 2 (State Gazette no. 87 of 24.10.2000, updated in State Gazette no. 27 of 11.03.2008). The rules of the good agricultural practice aimed at protecting water against nitrate pollution are approved by Order № ПД 09-799/11.08.2010 of the Minister of Agriculture and Food. In accordance with these documents, the input amount of nitrogen is 170 kg/ha per year (EU Nitrates Directive) in order to limit the pollution of soils and underground water caused by nitrates.

The results obtained from the model trials allows us to assess the environmental risk caused by the management of eco-technical systems for eggs. In contrast to the extensive, seasonal poultry farming, the production with intensive technologies is continuous, which also defines the dynamics of the risk – high risk in all seasons of the year, which is important with view of the mineralization intensity of organic substances in the manure that are most intensive during the summer, followed by the transitional seasons. One of the possibilities for mitigating such risk is to apply the standard of 170 kg/ha of nitrogen per year as set out in the Nitrates Directive at the stage of design and construction of eco-technical systems for eggs. Based on the details for distribution of nitrogen imported for the production of a unit of egg mass in the two flows of the secondary biological production, we can estimate minimum land areas to the egg farm, where the manure is utilized in accordance with cyclorama by years. By combining the egg production with biogas installation and mineralization degree of manure's organic substances of about 50% in the biosludge, the complete utilization of nitrogen imported with the manure occurs for 4 years /joint studies with Marinova et al, 2015/. Based on the two requirements: maximum admissible amount of nitrogen of 170 kg/ha and utilization period of 4 years, we determine the total area of agricultural plants divided into 4 equal plots where the input of nitrogen generated in the farm is rotated. Another option is to establish holdings for production of biological products that integrate 3 subsystems: eco-technical system for eggs, eco-technological module for biogas generation and agrocenosis with specific size where the generated biosludge is imported. A system for management of the nitrogen biochemical cycle is developed based on parameters obtained as a result of the modelling: amount of nitrogen contained in the manure from the specific eco-technical system – amount of nitrogen and data about non-protein nitrogen in biosludge – amount of nitrogen contained in the fodder plants within the agrocenosis. This determines the area of the agrocenosis. It depends on the mineralization degree during the manure processing. For the biosludge generation with maximum amount of nitrogen of 70 kg/ha an area for 4 years' cycle is dimensioned, however this area depends on the amount of nitrogen absorbed by the fodder cultures. This amount is determined within the frames of modelling in strict observance of Ordinance № 1/ 2015 on the biological production. The model allows precise balancing of the nitrogen movement along the entire eco-technological chain – eco-technical system for eggs – biogas generation – agrocenosis for biological production of fodder.

#### 4. CONCLUSIONS

The management of eco-technical systems for eggs is a system of knowledge and skills for ecologisation of technologies in stock-farming. The theoretical foundation lies on Vernadsky's doctrine about the noosphere /1945/ and the knowledge gained in the area of agroecology and economy, the combination of which is the scientific basis for a new kind of management covering a mix of economic and environmental priorities. In the wide concept of "environmental priorities" we emphasise on the environmental risk assessed in two aspects: human health and normal functioning of ecosystems /"health of ecosystems"/. As an algorithm of activities, ecologisation comprises the main stages of the systemic approach: theoretical knowledge, trials /the models we describe are of great importance/, analysis of results and application in practice. For the needs of the trials, we developed an integrated model of trophic chain that combines the two main modules of trophic relations at biocenotic level: trophic chain of pasture and detritus type. Technical means reproducing the processes in nature are developed: laboratory fermenter of cascade type and composting installation. Based on a comparative analysis we proposed a method for mathematical modelling of the anaerobic mineralization of organic substances in the manure in the biogas installation. Conducted studies show insufficient use of nitrogen from the ration for synthesis of specific egg whites and concentration of nitric compounds in the manure. The assessment of environmental risk shows that the amount of non-protein forms of nitrogen increases in both of the aerobic technologies and due to the installations' technical solutions, significant losses of nitrogen exist, whereas part of the nitric compounds transform into atmospheric pollutants. It is most expedient to use installations for generation of biogas for manure processing, where conditions for intensive and controlled anaerobic decomposition are established, where biogenic elements are completely preserved, where no greenhouse gas emissions exist, and except biosludge, which is an alternative of the highly energy consuming mineral manures, gas fuel /biogas/ is also generated.

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