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The articles appearing in this journal are indexed and abstracted in: EBSCO Publishing, Inc. and AGRIS (FAO). The journal is accepted to be indexed with the support of a project № BG051PO001-3.3.05-0001 “Science and business” financed by Operational Programme “Human Resources Development” of EU. The title has been suggested to be included in SCOPUS (Elsevier) and Electronic Journals Submission Form (Thomson Reuters).

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Bioconversion of nitrogen in an eco-technical system for egg production

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Abstract. The present paper aims to assess nitrogen circulation in an eco-technical system for egg production. The experiments were conducted in modelled conditions in an anthropogenic ecosystem of the “mesocosm” type, in which the unit of the bio-consumers and three eco-technological chains modelling the unit of the bio-decomposers are modelled: manure storing for decontamination /a recommendation on good farming practices/, composting and anaerobic decomposition in an installation for biogas production. A new criterion was implemented for the assessment of the chemical heterogeneity in the biogenic nitrogen cycle in the modified trophic chain – retention coefficient \( k \), which is defined as the ratio of nitrogen introduced into the system / nitrogen content in the feed: its quantity in the secondary biological production \( x \times 100 \). The chemical heterogeneity at the level of organisms /differences in the individual components of eggs/ and at the biocenotic level is established. The biogenic nitrogen cycle in the eco-technical chain for egg production is characterized by an uneven distribution in both products of the outflow. The largest amount of nitrogen is found in the egg whites \( (k = 0.49) \), while it decreases significantly in egg yolks \( (k = 0.17) \) and reaches \( k = 0.03 \) in the egg shell, a.k.a. heterogeneity on the level of organisms is established. The nitrogen compounds introduced through the feed ration are concentrated in manure \( (k = 25.33) \). Losses of nitrogen are established in two of the manure utilization technologies. The quantity of \( k \) in the compost is 20.32, a.k.a. the loss of nitrogen compounds is 19.8%. The biggest losses are found in manure storage; according to the recommendations on good farming practices \( (k = 18.82) \) or the reduction of nitrogen is 25.7% compared with fresh manure. Due to redistribution of the chemical elements /a significant part of C, H and O are included in biogas/, there is nitrogen concentration in bio slime – \( k = 35.85 \) or 41.5% more than in fresh manure. When separated nitrogen is concentrated in the liquid fraction \( (k = 31.19) \), while in the solid phase \( k = 4.67 \) is established.

Keywords: nitrogen, eco-technical system for eggs, anaerobic decomposition, organic fertilizers, compost

Introduction

The chemical heterogeneity of the biosphere proven by Vernadsky (1954) is the basis for important research practice in different areas, among which is management of the biogenic and toxic element cycle in agro-ecological systems and the related changes in quality of food, reduction and use of organic waste, etc. The object of this work is the circulation of a vital biogenic macroelement – nitrogen in anthropozoocenosis for egg production, which is a system of few components in structure and belongs to the eco-technical systems with broken cycle of matter and linearity of technological processes in its function. With the term “nitrogen” we define the biologically active nitrogen compounds in the biosphere. According to Odum (1975) and Ricklefs (1975), they are a defining structural component for all levels of organization of life, as they are the basis of egg white proteins, and “life is a form of existence of proteins” (Engels, quoted by Vernadsky, 1954). Ricklefs (1975), Schroder et al. (2003) and others, point out an important feature for the functioning of ecosystems and biosphere in characterizing the biogenic nitrogen cycle – differences in nitrogen retention in the biological systems of bodily (organs and tissues) and beyond-bodily (plant and animal species) levels of organization of living matter.

In order to achieve the main goal – maximum productivity at high economic efficiency, other significant features in the structure and functioning of the agro-ecological system are implemented: high population density, modifying the abiotic factors and regulation of biotic relationships, mandatory system of ecological control. In our experiments the trophic chain, in which the first unit is heterotrophic organisms – bio-consumers (the economically valuable population), is integrated with biotechnological chains of a detritus type of anthropogenic transformation which provides anaerobic decomposition of organic products – manure, which yields fuel gas (biogas) and a product which increases soil fertility (bio slime), with a biotechnological chain for anaerobic decomposition (composting) and a primitive model of a facility for substrate mineralization, which is recommended in the good farming practices (Welte and Timmermann, 1989).

The anthropogenic modification of the trophic chain of pasture type and the necessity for precise regulation of the movement of matter in the “bio-producers – bio-consumers – bio-decomposers” unit imposes a criteria search for assessing chemical heterogeneity at the level of the organism /in order to assess the quality and safety of manufactured secondary biological production/ and at the above bodily level /in this case for the assessment of organic fertilizers as a resource for improving soil fertility/. In forming the eco-technical system a trophic chain with a broken cycle of matter and a biotechnological chain for manure mineralization are formed. Therefore, control is concentrated on the units of the trophic chain of pasture type – bio-producers (feed) - bio-consumers (laying hens) - organic produce /eggs and manure/ and the biotechnological chain /secondary bio-technical produce /manure – biogas – bio slime or compost when composting/, respectively when storing manure (Baykov and Tyrawska, 1991; Paul and Beauchamp, 1995; Kostadinova and Petkov, 2012). As a general criterion for the assessment of nitrogen’s chemical heterogeneity we apply a quantitative criterion “retention coefficient” \( (k) \), which similarly to the criteria related to bioaccumulation – concentration clark, safety clark, etc. allows control and regulation of matter movement in the anthropogenic ecosystem for egg production in order to reduce the consequences of the technological process linearity and to guarantee the quality of the secondary biological produce used as food for man (eggs), (Baykov et al., 2005). The proposed

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quantitative criterion (k) is indicative for the degree of accumulation of organic nitrogen compounds in the secondary biological produce, which is a key indicator of its biological value.

**Material and methods**

Our research was conducted in modelled conditions in an eco-technical system for the production of eggs in which the trophic chain of pasture type is modified, with broken cycle of matter and linearity of technological processes due to which waste products are accumulated.

The experiments have been carried out in modelled conditions in anthropogenic ecosystem of the “mesocosm” type under Odum’s classification (Odum, 1986), which includes two models: model of anthropozooocenosis with a trophic chain of pasture type and two types of models of trophic chains of a detritus type, biotechnological chain for anaerobic decomposition of the manure organic matter /biogas production/ and for aerobic decomposition /composting/, as well as a scale model for the storage and mineralization of manure recommended in good farming practices. The model of a livestock farm is realized in an anthropogenically modified ecotope – air-conditioned sectors of an experimental base near Kosţinbrod. The abiotic parameters of the habitat are in accordance with Decrease No 44 of the Ministry of Agriculture and Food, as follows: air temperature (16 – 22°C), relative humidity (50 – 75%), air velocity in the living area (0.2 – 0.5 m/sec), light intensity (40 lux), toxic gas content – carbon dioxide (0.1%), ammonia and hydrogen sulphide – traces. The birds are watered with water from a central water source which meets the requirements under Regulation No 9/2001 of the Ministry of Health. The nitrate content in the drinking water is under 10 mg/L and therefore this source of nitrogen has not been taken into account when determining (k).

The studies have been conducted using 80 laying hens in 4 equal groups of the ISA-Brown breed, all 36 weeks of age. The biotic factors are regulated by the application of the established in this technology prevention programs and by regulation of the abiotic factors /the intensity of air exchange/ with view of limiting microbial air pollution. The fowls are reared without bedding at a density of 6 hens/m² floor area. The laying hens are fed with a feed containing 18% crude protein, 0.44% assimilable phosphorus, 3.83% calcium, 0.91% lysine, 0.76% methionine + cysteine and metabolic energy 2759 kcal/kg of the feed.

The second module /biotechnological chain for biogas production/ includes modelling of anaerobic biomass decomposition /AD/ in a laboratory cascade fermenter developed by us and optimized for the technological processes through mathematical modelling. The technical solution allows for conducting an experiment with 6 simultaneous substrates as an opportunity for daily control of the anaerobic decomposition by sampling at the input and output of each module of the cascade.

To optimize methane fermentation we applied the model of Chen and Hashimoto (1979), which is used by a number of authors in a handling laboratory, pilot and industrial biogas installations. It is applied in a very wide range of values of the individual variables, as well as in various substrates (Chen and Varel, 1980). On the basis of Chen and Hashimoto (1979) and Chen and Varel (1980)'s models we developed a modified form of the model and a program for optimization analysis, the equation has the following form:

\[
P = B_v \cdot 2.6 \cdot 10^3 \left(1 - \frac{K}{\mu m \theta - 1 + K}\right)
\]

\[
Y_v = \left(\frac{P}{2.6 \cdot 10^3}\right) \cdot \left(\frac{S}{\theta}\right)
\]

\[
V = \theta \cdot \frac{2.6 \cdot 10^3}{S_1}
\]

\[
Q = B_v \cdot 2.6 \cdot 10^3 \left(1 - \frac{K}{\mu m \theta - 1 + K}\right)
\]

where the variables are as described in the model and in addition: \(P\) – the total methane yield in a fermenter recasting manure derived from a number and category of birds, listed in a table; \(V\) – fermenter volume; \(Q\) – degree of decomposition of organic matter (in %); \(Y_v\) – technological methane yield – dm³ CH₃/ dm³ of ferment; \(B_v\) – maximum methane yield in mineralization of a unit organic matter of the substrate dm³/ CH₄/gVS. \(S_1\) – concentration of the organic matter of the substrate – gVS/dm³. \(\theta\) – exchange period (in days); \(K\) – kinetic constant without dimension; \(\mu\) – the maximum specific growth rate of the microbial population – day⁻¹.

The model's modified form that we applied and the program for optimization analysis allow to determine the total yield of methane (dm³ CH₄/day), the technological methane yield (dm³ CH₄/dm³/day), the degree of decomposition of the organic matter of the substrate (%), as well as the reactor volume in m³ at different values of the variables in the technological regime, relative to a farm for rearing 5000 laying hens (a main type of farm for biological egg production). The program for computer analysis allows for optimization of designing the installation and development of an optimal technological regime.

The data for the quantity of the manure are taken from the research by Baykov and Tyrawska (1991). In this current model we accept as criteria for optimization the following: maximum mineralization of the organic matter in the substrate /ecological criterion = criterion P /respectively maximum total gas production or factors /the intensity of air exchange/ with view of limiting microbial air pollution programs and by regulation of the abiotic variables in the technological regime, relative to a farm for rearing 5000 laying hens (a main type of farm for biological egg production). The program for computer analysis allows for optimization of designing the installation and development of an optimal technological regime.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Criteria</th>
<th>(P)</th>
<th>(Y_v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, T</td>
<td>°C</td>
<td>33</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Exchange period,</td>
<td>Days</td>
<td>15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Substrate concentration, S0</td>
<td>GVS/1</td>
<td>39</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Load</td>
<td>GVS/day</td>
<td>2.6</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Useable capacity, V</td>
<td>m³</td>
<td>32.5</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>Total methane yield, P</td>
<td>m³/day</td>
<td>21.4</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>Technological methane yield, YV</td>
<td>CH₄/day</td>
<td>0.65</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Degree of degradation, Q</td>
<td>%</td>
<td>49.27</td>
<td>32.76</td>
<td></td>
</tr>
</tbody>
</table>
The second variant of the biotechnological system is realized in a laboratory-based composting installation, described by Zaharinov (2013). The aeration intensity and the idle time of the substrate are in accordance with the methodology of Baykov and Zaharinov (2005). The manure storage module is a scale model of manure storage made from durable plastic, where the shelf life is 180 days. The laboratory tests were conducted using the following algorithm:

Eggs are collected and tested daily. Egg white, yolk and shell are separated and individually weighed. The samples are stored at 0°C until the time of testing (every 10 days).

Daily samples were taken from the manure (both fresh and stagnant), bio slime and compost and periodically (every 10 days) from the feed and drinking water, which were tested by standardized methods, as follows:

- Determining mass in a fresh state (through weighing).
- Determining the environmental response (pH) - BDS EN 12176:2000.
- Determining mineral nitrogen forms: ammonium (NH₄-N) and nitrate (NO₃-N).


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

Results and discussion

The results for the chemical heterogeneity of nitrogen in an anthropogenic ecosystem for the production of secondary biological produce (eggs) are presented in Table 2 and Figure 2. In applying the criterion k, substantial differences in nitrogen retention in the two main branches of the secondary biological produce were discovered. The principal amount of nitrogen is concentrated in manure, which contains 25.33% of the introduced amount of nitrogen (k = 25.33), while in eggs the nitrogen retention is respectively 0.17% in the yolk (k = 0.17), 0.45% in the whites (k = 0.45) and 0.03% (k = 0.03%) in the shell. The studies show significant differences in the retention of nitrogen in the different

Table 2. Movement of nitrogen in the trophic chain of the eco-technical system for egg production (n = 6)

<table>
<thead>
<tr>
<th>N</th>
<th>Element</th>
<th>Measure mg/100g of dry matter quantity</th>
<th>Retention coefficient (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mixed fodder</td>
<td>2880±18.20</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Egg yolk</td>
<td>4.77±0.82</td>
<td>0.17</td>
</tr>
<tr>
<td>3.</td>
<td>Egg whites</td>
<td>13.17±1.14</td>
<td>0.45</td>
</tr>
<tr>
<td>4.</td>
<td>Egg shell</td>
<td>0.92±0.42</td>
<td>0.03</td>
</tr>
<tr>
<td>5.</td>
<td>Fresh manure</td>
<td>729.3±38.90</td>
<td>25.33</td>
</tr>
<tr>
<td>6.</td>
<td>Dry manure</td>
<td>542.2±41.90</td>
<td>18.82</td>
</tr>
<tr>
<td>7.</td>
<td>Compost</td>
<td>585.19±30.90</td>
<td>20.32</td>
</tr>
<tr>
<td>8.</td>
<td>Bio slime total</td>
<td>1032.4±48.10</td>
<td>35.85</td>
</tr>
<tr>
<td>10.</td>
<td>Bio slime solid fraction</td>
<td>134.21±28.20</td>
<td>4.67</td>
</tr>
</tbody>
</table>
studies, based on a modified by us methodology by Vernadsky (1954) for the assessment of the chemical heterogeneity of the biosphere based on Clark’s teachings for the chemical heterogeneity of the lithosphere. For almost 40 years (the period of 1999 – 2024) and on the basis of more than 5000 chemical analyses, Clark proves the average content of the main ten chemical elements common in the surface layer (up to a depth of 16 km) of the lithosphere (Dobrovolysky, 1998). In 1934 Fresman offers quantitative assessment criterion for this heterogeneity. The Clark parameters, including the amount of nitrogen (Reylly, 1980; Schroder et al., 2003).

When calculating /k/ of mass of the studied lithosphere sample to the average content of that the analysis on one trophic level in this case the level of the anthropozoocenoses for egg production the manure as a raw material with strictly controlled amount of nitrogen surpluses introduced through the ration are not utilized fully from the economically valuable population and are concentrated in the manure. From this feature stems the need for interpretation of the obtained results it should be borne in mind that the analysis on one trophic level in this case the level of the anthropozoocenosis type “macrospace” which have a technological path for their usage. This is the applied principle in the determination of (k) of nitrogen in an eco-technical system for the production of eggs. As with Verdansky (1954)’s research, the used criterion reflects nitrogen retention in the bioconsumer unit, a.k.a. the end result of the anthropogenically formed nitrogen cycle, as the (k) criterion allows for a quantitative assessment. When calculating /k/ the numerator is the nitrogen in the tested substrate (yolk, manure, etc.) in 100g of dry matter; the amount of nitrogen per 100g dry weight of feed x 100. The denominator is Clark’s average content of that the element in the crust. When K is above 1 we talk about concentration, if it is less than 1 we talk about diffusion.

Based on the obtained results a conclusion should be drawn that the enrichment of the ration with nitrogen compounds (amino acids and proteins), regardless of the fact that it stimulates productivity of populations of highly productive breeds and their hybrids, it is ineffective from the point of view of biogeochemistry, to due the fact that the extremely high content at the input of the trophic level of the bioconsumers is the reason for the accumulation of a large amount of nitrogen compounds in manure (the second major component of the secondary biological produce). It should be emphasized that the nitrogen compounds are involved in the metabolism of the fowl’s body, as only a small part turns in specific structural proteins, as well as in egg whites, while the others serve as energy sources and through pre-animation and de-animation are included in Krebs’ cycle for energy release. In de-animation nitrogen compounds are released which then accumulate in manure and this is one of the reasons for the high nitrogen content in excrements. The conclusion is that in the egg, whose chemical composition is relatively constant (homeostasis is required for the embryo), the respective ratio between the nutrients is also maintained. This ratio is predetermined by evolution. The additional amounts of nitrogen in the rations stimulate the birds’ productivity (amino acids are an accessible source of energy), but this is not associated with changes in the nitrogen content of eggs. The conclusion is that similarly to the energy subsidies in the anthropogenic ecosystems which Odum (1996) writes about, in the anthropozooocoenoses for egg production the nitrogen surpluses introduced through the ration are not utilized fully from the economically valuable population and are concentrated in the manure.

Table 3. Agrochemical and chemical characterization of solid and liquid of hen manure – samples from field studies of anthropozooocoenosis type “macrospace” (n = 6)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Fresh manure</th>
<th>Dry manure</th>
<th>Compost</th>
<th>Bio slime total</th>
<th>Bio slime liquid fraction</th>
<th>Bio slime solid fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.66</td>
<td>7.54</td>
<td>7.84</td>
<td>7.18</td>
<td>7.28</td>
<td>7.11</td>
</tr>
<tr>
<td>Dry matter, %</td>
<td>48.16±1.14</td>
<td>40.14±1.84</td>
<td>36.24±2.64</td>
<td>4.08±0.64</td>
<td>2.71±0.22</td>
<td>1.44±0.14</td>
</tr>
<tr>
<td>Organic C, %</td>
<td>30.72±1.18</td>
<td>20.41±1.44</td>
<td>19.22±1.64</td>
<td>15.12±0.84</td>
<td>10.12±2.69</td>
<td>4.66±1.22</td>
</tr>
<tr>
<td>Total N, %</td>
<td>7.14±0.86</td>
<td>5.41±0.64</td>
<td>6.06±0.44</td>
<td>9.86±0.24</td>
<td>8.22±0.66</td>
<td>1.26±0.32</td>
</tr>
<tr>
<td>Removable N–NH₄, %</td>
<td>0.81±0.34</td>
<td>0.81±0.26</td>
<td>1.24±0.22</td>
<td>0.84±0.19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N–NO₃, %</td>
<td>3.96±0.86</td>
<td>24.22±0.84</td>
<td>36.18±2.64</td>
<td>44.48±3.64</td>
<td>34.60±4.64</td>
<td>10.10±1.64</td>
</tr>
</tbody>
</table>

To clarify the issue we conducted additional studies in which the movement of nitrogen within the unit: bioconsumers (poultry, manure respectively) - bioproducers – processed product/bio slime, compost, stagnant manure/, the results of which are presented in Table 3 and Figure 3. The conducted studies on the nitrogen cycle in an eco-technical system for the production of eggs chain show some alarming for nature's health consequences from the currently applied technologies for manure utilization. The most popular technological solution is the storing of manure for 180 days, which is necessary for decontamination and partial mineralization of the manure according to the authors of the technology. When storing manure for 180 days, nitrogen losses are 24.3%. Those losses are related to emissions in the atmosphere mainly of ammonia and other gases from the vital activity of the microbial community in manure. During storage the amount of energy contained in manure is reduced, which translates into reduction of organic carbon by 33.6%. These results give ground for reassessment of the wide-spread practice for prolonged storage of manure with a view to its mineralization and microbial decontamination, as the search for environmentally friendly solutions is recommended, which guarantee storage of the biogenic elements and the energy contained in manure.
The closest technology to the ongoing processes in natural ecosystems of mineralization of organic matter of the bioproducers unit is composting. Due to redistribution of the chemical elements in the compost, the nitrogen losses are relatively small (14.2%) at established emissions of ammonia and other toxic gases in the atmosphere. In this case the significant quantitative losses in the form of nitrogen-containing gases are compensated by the redistribution of the chemical elements and especially of significant carbon dioxide emissions. The assessment of this technology is that in eco-technical systems – that is in intensification of the processes of egg production – it is environmentally inappropriate. Even though it doesn’t introduce energy from the outside, significant energy losses are established at the expense of oxidation of the organic matter in manure – so that the amount of carbon in the compost is 34.4% less in comparison to fresh manure, which deteriorates the quality of the compost as a source of energy for soil biocenoses.

The biotechnology for the anaerobic decomposition of organic waste has significant advantages over the other two technologies. Two products are obtained (gas fuel – biogas) and a product which increases soil fertility (bio slime). The redistribution of the chemical elements in bio slime in the production of biogas is associated with the release of significant amounts of carbon, hydrogen and oxygen in the biogas (a mixture of CH₄ + CO₂). The process occurs in a sealed environment and as a result the relative amount of nitrogen in the bio slime increases, (k) reaches 35.85 (at 25.55 in fresh manure). Only with this technology there is no loss of energy – carbon reduction of 50.8% is due to its inclusion in the methane of the gas fuel, which finds a variety of applications, but it is the only technological solution in which the mineralization of the manure does not require the introduction of energy from the outside.

The distribution of the nitrogen compounds in the separation of the bio slime (which contains about 4% dry matter in our experiments) has an important practical significance: 83.3% of the nitrogen compounds are contained in the liquid fraction, while 12.8% in the solid one. The majority of carbon is also contained in the liquid fraction (Table 3). This distribution gives grounds to recommend the use of the solid fraction as fuel (after drying and pelleting), as it is not a major source of nitrogen and energy. The presence of a large amount of nitrogen compounds in the liquid fraction is an indicator for the real danger of pollution of surface and subterranean waters at infiltration of liquids near livestock farms or direct discharge of the liquid fraction into the water.

**Conclusions**

For assessing the chemical heterogeneity in the biogenetic cycle of nitrogen in an eco-technical chain for eggs a new criterion was implemented – retention coefficient (k), which is determined on the basis of the nitrogen introduced into the system (the nitrogen content in the feed) and its quantity in the secondary biological productive. Chemical heterogeneity on the level of the organism is established (differences in the different components of eggs) and on the biocenotic level. With the movement of nitrogen in the eco-technical chain for egg production, an uneven distribution of nitrogen in both products of the output stream was established. In the nitrogen outflow (k) in eggs, the amount of nitrogen is the highest in the egg white (k-0.45) and it decreases significantly in the yolk (k – 0.17), as it reaches k – 0.03 in the egg shell. The nitrogen compounds introduced through the feed concentrate in the manure (k = 25.33). Nitrogen losses are established in two of the technologies for manure utilization. The quantity of (k) in compost is 20.32, i.e. the nitrogen compound losses are 19.8%. The biggest losses are found in storing the manure according to the recommendations on good farming practices (k = 18.82) or by 25.7% compared to fresh manure. Due to redistribution of chemical elements (a significant part of C, N and O are included in the biogas) there is nitrogen concentration in the bio slime – k = 35.85 or by 41.5% more compared to fresh manure. When separating the nitrogen is concentrated in the liquid fraction (k = 4.67) it is settled.
Acknowledgements

The present development is a result of the fulfillment of a contract DFNI-E01/3 of 27/11/2012, funded by Fund for Scientific Investigations to whom we express our gratitude.

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A. Stoilova, I. Saldzhiev

Influence of the direction of crossing on heterosis and transgression events in relation to the length of the vegetative period of Burley tobaccos variety group
Y. Dylgerski, T. Radoukova, L. Dospatliev

Nutrition and Physiology

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Feeding value estimation of spring forage pea (*Pisum sativum* L.) in organic cultivation
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Production Systems

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I. Effect on grain yield from wheat
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Crop relationship "yield-evapotranspiration" for green bean
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Agriculture and Environment

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D. Petrova, D. Gerdzhikov

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