# NEW SELENIUM-ENRICHED FODDER YEASTS AND THEIR APPLICATION IN RATIONS OF LAYING HENS

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**Introduction**

The growing demand for selenium-containing food and folder is determined by its role to provide normal life activities of all biological creatures, including humans and animals. Selenium (Se) normalizes liver function, shows antioxidant, immunomodulating, detoxifying properties, is actively involved in generation of active sites in several enzymes. The lack of selenium is responsible for metabolic disbalance, retarded growth, degenerative changes in muscle and hepatic tissues, cardiomyopathy, reproductive dysfunction. The shortage of this microelement on the cellular level disrupts integrity of cell membranes, reduces enzyme activity, provokes intracellular calcium accumulation, impairs metabolism of amino- and ketoacids, suppresses energy generation processes, etc. This, in turn, causes such pathologies as cancer, Keshan or Kashin-Beck diseases, muscular syndrome, often resulting in lethal outcome [1–4].

Alimentary products of plant and animal origin as well as water serve as sources of selenium for humans and animals. Therefore, depending on the geochemical factor, the content of the microelement in the consumed food and feed products determines the level of its physiological uptake by humans and animals. Taking into account that selenium consumption dose recommended by World Health Organization varies from 40 to 200 µg per day, its deficiency on the global scale affects 0.5 to 1.0 bln people. Belarus is distinguished by the moderate selenium deficit requiring permanent supply of Se-enriched premixes. Their introduction into feed rations solves the problem of microelement deficiency not only in animals, but also in the human body through the consumption of animal products enriched with this microelement.

Inorganic selenium widely used to date in feed formulas is not efficiently assimilated, displays low biological value, high toxicity, in overdoses may induce toxicoses or even lethal cases. On the contrary, organic selenium compounds are less toxic, hence they are more suitable for incorporation into tissues [5–6]. The most readily available, economically grounded and ecologically safe method of commercial production envisages transformation of inorganic selenium in microbial cells into organic Se varieties.

Among selenium-accumulating microorganisms, bacteria of genera *Lactobacillus*, *Propionibacterium*, *Bacillus*, mycelial fungi of genus *Aspergillus*, basidiomycetes of genera *Ganoderma*, *Agaricus*, *Grifola*, *Hericium* and yeast-like fungi may be pointed out.

The latter are represented by natural selenium-adapted cultures *Candida utilis* CUM, *Hanseniaspora uvarum* (*Hansenula vinifera*), *Saccharomyces cerevisiae* TZJM and mutant strains subjected to chemical and physical mutagenesis (*Candida glabrata* FXY-4, *Candida utilis* S1204), as well as molecular genetic modifications (*Rhodotorula glutinis* X20).

The world market of Se-containing yeast abounds in plenty of manufacturers dominated by the most influential corporate players, like Assotiated British Foods Inc. (UK), Archer Daniels Midland Company (US), Alltech Inc. (US), Cargill (US), Angel Yeast Company (China), Chr. Hansen (Denmark), Lesaffre (France), etc. In the countries of Eurasian Economic Community microbiological manufacturing of selenium-enriched products has not been launched so far. Currently the project aimed at elaboration of pilot-plant technology of producing Se-enriched fodder yeasts based on strain *Candida stellimalicola* 4-ASe adapted to this microelement is nearing the completion at the Institute of Microbiology, National Academy of Sciences of Belarus [7].

The aim of the present investigation is the comparative evaluation of the effect of sodium selenite and Se-enriched fodder yeasts on productivity of laying hens and egg quality.

**Materials and methods**

Feed yeast culture fortified with selenium in concentration 2,000 mg/kg, was based on strain *Candida stellimalicola* 4-ASe adapted to this microelement. The strain was deposited in Belarusian collection of non-pathogenic microorganisms under registration number BIM Y-350 D.

The studies were carried out at Genestock division of the 1st Minsk poultry factory and at Experimental research station for poultry breeding. To conduct experiments 3 groups of local cross-bred laying hens were chosen, each comprising 30 heads. The groups were formed in accordance with the principle of similarity in origin, age, gender, live weight. The fowl was kept in individual cages. Rearing density, light, temperature, humidity regimes, and other breeding parameters matched the standards set for the tested cross line.

Laying hens of the first control group were fed the composite ration balanced in major nutrients and sodium selenite (200 mg Se / t) added into standard premix formula. The fowl from 2 and 3 test groups was fed composite fodder fortified with Se-enriched yeast in amount 0.1 and 0.15 g/t (200 and 300 mg Se / t), respectively. The test batches of composite feed were fabricated by Alnikorprodukt Vertelishki (Belarus).

The generally recognized methods were used to determine survival of chickens, live body weight, fodder consumption, egg-laying capacity, egg weight, feed expense per 10 eggs, fodder spent to produce 1 kg of egg mass, the yield of egg mass per 1 fowl, egg category, morphological composition of eggs, the contents of vitamin A and carotenoids in egg yolk.

Statistical processing of experimental data was performed by regression analysis (Student's test) using Microsoft software.

**Results and discussion**

The results of feeding trials demonstrated that supply into the rations of both sodium selenite (200 mg Se / t) and selenium-fortified yeast (200 and 300 mg Se / t) did not affect viability and productivity of laying hens. In all experimental groups egg- laying capacity per average hen did not vary significantly: 56.6 eggs in the first control group versus 56.4 and 56.8 eggs in the second and third test groups. When selenium dose supplied into the rations with fodder yeast rose from 200 to 300 mg Se / t of composite feed egg productivity increased by 0.4%. The intensity of egg production in chickens of the 1 control group equaled 71.7%, whereas in the 2 and 3 groups it varied from 71.4 to 71.9%.

Irrespective of selenium source in the rations, the hens were eating the fodder readily and almost in the same amount. The average daily feed consumption in all groups was identical (126.9 g), as well as fodder expense (1.77–1.78 kg) per output of 10 eggs.

It should be noted, however, that Se-enriched yeast exerted stronger effect on the weight of produced eggs in comparison with added sodium selenite (Table 1)

**Table 1. The data of morphological egg examination**

| Parameters | Groups | | |
|---|---|---|---|
| | 1 (control) | 2 (test) | 3 (test) |
| Egg weight, g | 53.6±1.15 | 54.8±1.43 | 56.2±0.82 |
| Shape index | 76.1±0.50 | 76.2±0.54 | 76.9±0.48 |
| Haugh units | 77.9±2.40 | 79.9±4.80 | 83.0±2.18 |
| The ratio of albumen weight to yolk weight | 2.3±0.06 | 2.5±0.06 | 2.4±0.05 |
| Shell thickness, µm | 353±6.7 | 346±9.5 | 373±5.98* |
| Albumen index | 0.082±0.005 | 0.089±0.007 | 0.085±0.005 |
| Yolk index | 0.401±0.007 | 0.423±0.007* | 0.410±0.005 |
| Shell weight, g | 5.9±0.23 | 5.9±0.22 | 6.4±0.17 |
| Yolk weight, g | 14.4±0.30 | 14.3±0.39 | 14.9±0.26 |
| Albumen weight, g | 32.9±0.84 | 34.6±0.99 | 34.8±0.59 |

Note: *the difference between the parameters of the control and test groups is authentic at $P \leq 0.01$

In contrast to chickens from the first control group this parameter in hens of the 2 and 3 groups was higher by 2.9–3.5 % ($P \leq 0.001$). Likewise, the yield of the egg mass per each hen in the 2 and 3 groups was higher by 2.7–3.4% if compared with the control parameter. The increase of average egg weight in the 2 and

3 groups accompanied by the identical feed consumption resulted in the reduced feed expense (2.3–3.5%) to produce 1 kg of egg mass. The fact that substitution of organic selenium for inorganic analog in hen rations enhanced egg weight and saved fodder spent to produce 1 kg of egg mass was also stated by other researchers (8–9).

When sodium selenite was replaced with Se-enriched yeast in composite fodder, the rising tendency was recorded for the parameters characterizing egg incubation properties (table 1). For instance, eggs laid by the hens from 2 and 3 groups were distinguished by elevated Haugh units (2.6–6.5%), albumen index (3.7–8.5%), and yolk index (2.2–5.5%) (P≤0.01 for the 1 control and 2 test groups).

The similar (P≤0.01) beneficial effect of yeast fortified with selenium (300 mg Se / t) on thickness of egg shell was revealed in the 3-test group – this parameter equaled 373 µm; exceeding by 5.7% the similar parameter in hens of the 1 control group. As a result, egg shell mass in chickens of the 3-test group augmented by 8.5%. Favorable impact of organic Se compound in feed additive on morphological characteristics of eggs, namely on shell thickness was also recorded by other researchers [9].

The indexes of egg weight evaluated in the course of morphological examination and gravimetric assessment of gross egg yield were comparable in the 1 control and 2 and 3 test groups of laying hens. Noteworthy that weight of eggs produced by fowls in 2 and 3 test groups surpassed by 1.2–2.6 g that in the control group as a consequence of increased protein content in eggs by 5.2–5.8% (from 32.9g to 34.6–34.8 g) resulting in enhanced albumen to yolk mass ratio (from 2.3 to 2.4–2.5).

Upon supplementation of rations with Se-enriched yeast premixes concentration of vitamin A in eggs of hens from the 2 and 3 test groups rose by 1.7–2.4%, while the level of carotenoids tended to grow by 4.2–6.4% as compared with the control. The maximum amount of vitamin A and carotenoids was detected in eggs of poultry from the 2-test group receiving 200 mg Se / t of composite feed.

Distribution of eggs collected from fowl in all studied groups into quality categories is reflected in table 2.

**Table 2. Egg distribution into categories**

| Egg category | Egg distribution into categories, %: | | |
|---|---|---|---|
| | 1 control group | 2 test group | 3 test group |
| **Supreme** | 0 | 0.6 | 0.3 |
| **Premium** | 0.3 | 0.6 | 0.6 |
| **First** | 23.7 | 33.1 | 31.1 |
| **Second** | 68.9 | 64.8 | 65.0 |
| **Small** | 7.1 | 0.9 | 3.0 |

It is evident from table 2 a significant decline in the number of low-value eggs produced by the hens from the 2 and3 test groups: in comparison with the 1 control group the proportion of small eggs fell from 7.1 to 0.9–3.0%, the ratio of the eggs of the second category dropped from 68.9 to 64.8–65.0%. The share of eggs in top price categories increased considerably: the first category from 23.7 to 31.1–33.1%, premium category from 0.3 to 0.6%, supreme category from 0 to 0.3–0.6%.

Our findings are partially correlated with literature data testifying to the improved physical (survival rate, bodyweight gains, egg productivity) and reproductive (fertility, egg hatchability, puberty period) parameters of poultry, upgraded quality of farm products (enlarged weight of eggs, higher protein and selenium contents) depending on age, concentration and duration of Se-containing yeast supply [10–14].

**Conclusions**

Using a novel microbial strain *Candida stellimalicola* 4-ASe, the first in Belarus pilot-scale technology of manufacturing fodder yeast fortified with selenium was elaborated. A test batch of the new feed product containing 2000 mg Se / kg was produced and passed successful large-scale trials.

It was found that introduction into the rations of both sodium selenite and selenium-enriched yeast did not significantly affect viability and productivity of laying hens. However, addition into chiken feed formulas of selenium-enriched yeast (200 and 300 mg Se / t of composite fodder) in contrast to sodium selenite supplement (200 mg Se / t) improved morphological characteristics (Hough unis, albumen and yolk indexes, shell thickness) and raised protein ratio in eggs as well as concentrations of vitamin A and carotenoids. Moreover, in laying hens nourished with selenium-enriched yeast, the yield of small and second category eggs tended to decrease with concomitant rise in the share of eggs representing the first (from 23.7 to 31.1–33.1%), premium (from 0.3 to 0.6%) and supreme (from 0 to 0.3–0.6%) categories.

Scaling up the process of manufacturing Se-containing feed additives based on a novel yeast strain *Candida stellimalicola* 4-ASe will enable to broaden the range of fodder commodities launched onto the market. Their applications relying on the analyses of biochemical blood tests, examination of intestinal microbiota of laying hens, efficiency of selenium incorporation in eggs, will promote enhanced yields, quality and profitability of poultry products, fabrication of foodstuffs preventing diseases provoked by selenium deficiency.

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