ОРИГИНАЛЬНАЯ СТАТЬЯ

SELENOCYSTINE SYNTHESIS AND PROSPECTS OF ITS UTILIZATION IN CORRECTION OF SELENIUM DEFICIENCY

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ABSTRACT. Inorganic compounds of selenium, selenium fortified yeast and selenium containing xenobiotics widely used for correction of selenium deficiency in domestic animals and human beings possess several serious drawbacks. L-selenocysteine (an oxidized form is selenocystine) demonstrating full physiological compatibility, anticancerogenic, antiradical, immunomodulating and protective functions is a prospective compound both for correction of selenium deficiency and biological activity. L-selenocystine synthesis was developed in Penza agricultural academy (Pat. RF №2537166). Capillary electrophoresis of the compound revealed the identity with Sigma L-selenocystine. To demonstrate potential abilities of L-selenocystine an experiment was achieved on broilers cross "Ross 308". Utilization of 0.3 g Se/t of feed (as a selenocystine) with OMEC premix resulted in 1.35 % increase of broilers biomass and a 0.88 % reduced cost of feed compared to control group and a group of broilers with selenite consumption (Se – 0.3 g/t). Analysis of selenium content in cnemis revealed that 0.3 g Se/t of selenocystine increased cnemis selenium content up to 0.24 mg/kg – the same as in the group with sodium selenite (Se – 0.3 g/t) being 0.07 mg/kg greater than in the control group.

KEYWORDS: selenium, selenocystine, synthesis, capillary electrophoresis, broilers, growth promotihg effect, daily weight gains.

Selenium (Se) deficiency is known to be one of the reasons of various chronic diseases in human beings. Besides this increased Se concentrations significantly affect antioxidant, immunological and detoxification systems, decreasing the resistance of an organism to infections.

Since Se essentiality to mammals has been proved, many attempts have been achieved in the determination of Se-containing compounds with optimal properties: physiological compatibility, relatively low toxicity and lack of prooxidant action on vitamins and other food additives and easy for standardization. Besides this such a compound should be cheap, simple in production, stable during storage.

Widely used inorganic forms of Se: selenates and selenites – possess high toxicity, intensive interaction with reductants (cystine, reduced glutathione) with formation of elemental Se of low biological activity (Ip, 1985).

Biotransformation of inorganic Se forms to organic ones during yeast and algae biofortification are the most widespread in the world. Nevertheless Se-enriched yeast and algae are not strictly identified compounds: besides residual sodium selenite there are several Se containing peptides and amino acids. Yeast preparations are shown to contain not less than 10–39% of inorganic Se forms (Derabina et al., 2006; Galochkin, Galochkina, 2011).

Other possibility of Se status optimization is utilization of organic Se derivatives produced via chemical synthesis. Such compounds may be divided to two groups: analogs of natural compounds found in food (Se-amino acids, Se-containing sugars) and xenobiotics (chemical compound not participating in natural biotic turnover).

Several preparations may be attributed as Se containing lipophilic xenobiotics: DAFS-25, selenopyrane and Ebselen. The active form of the first one is diacetophenonyl selenide ("Sulphate" corporation, Saratov) (Drevko, 2001). Selenopyrane is 9-phenyl-sym-nona-hydro-10-selenoanthraene, SP-1) (Blinokhvatov, 1993). Ebselen (2-phenylbenzoselenazol-1,2-3(2)-on) is produced in Germany as a pharmacological preparation (Sies, 1993). It should be noted that many questions of biological effect of DAFS-25 and other Se-containing xenobiotics on living organisms are poorly investigated.

We have demonstrated that DAFS-25 reacts with SH- groups of cystein, glutathione and proteins forming red elemental Se (Se^0), which is precipitated on cell walls (Poluboyarinov et al., 2009). Another reaction product is acetophenon.

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Selenopyran also forms elemental Se in acidic-catalyzed hydrolysis reaction (Poluboyarinov, 2011). Formation of elemental Se may be a reason of relatively low toxicity of these compounds.

Taking into account these facts investigation and utilization of new organic Se compounds in feeding industry seems to be of great importance.

The most prospective field of research in human and animal Se status optimization is utilization of Se-containing L-amino acids – Se-methionine and Se-cystein, produced via synthesis. The benefit of such a method is total physiological compatibility of these compounds. L-Amino acids seem to be ideal Se donors due to the ability to participate in natural metabolism.

The main chemical forms of Se in food products are Se-methionine (SeMet) and Se-cystein (Sec) and its oxidized form – Se-cystine (Sec-Sec).

Due to high similarity of physico-chemical properties of SeMet and Met they are able to replace each other in tissues proteins via specific to Met metabolic way. That is SeMet is incorporated in proteins non-specifically forming Se-pole. Consequences of such incorporation are not fully understood.

Shortcoming of SeMet is its rather high toxicity comparable with the toxicity of inorganic Se forms and also complex and expensive synthesis.

Sec-Sec should be considered the most prospective organic form of Se for correction of Se-deficiency.

Sec is the 21st essential amino acid, which biosynthesis is encoded by UGA terminating codon in conditions of the existence of stimulating nucleotide consequence (Berry et al., 1993). This is the most important natural form of Se because other derivatives are either intermediates in its biosynthesis or its metabolites.

Biological functions of Sec include protection against γ-radiation causing a decrease of DNA damage (Kunwar et al., 2011) and also antihaemolitic and antiradical activity (Kumar et al., 2011). Besides this Sec is shown to express a powerful protection against CCl_4 liver cells damage in rats (Uzma et al., 2011).

It should be also noted that Sec demonstrates anti-carcinogenic activity, inhibiting cell proliferation of breast adenocarcinoma MCF7 via induction of cell cycle and apoptosis locking (Chen, Wong, 2008a) and causes apoptosis of human melanoma cells A375 (Chen, Wong, 2008b). Increase of the amount of active oxygen forms (AOF) and breakage of DNA in cancer cells (MCF7 and HepG2) is registered contrary to normal human fibroblast cells Hs68 where AOF amount increase is not registered (Chen, Wong, 2009).

Besides this Sec is shown to possess immunomodulating activity – increasing functional activity of B-cells and causing an increase of secretion value of immunoglobulin M by 172 % (Stabel et al., 1991).

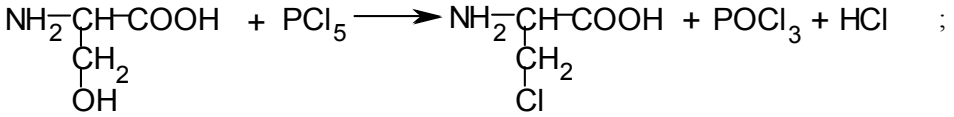
Sec toxicity (LD_{50} 35.8 mg/kg) is shown to be significantly lower than toxicity of SeMet (LD_{50} 4.3 mg/kg) and sodium selenate (LD_{50} 3.5 mg/kg) (Sayato, 1993). Other results of Sec toxicity determination give values of 76 mg/kg (oral administration, mice) (Klug, 1953) and 8.46 mg/kg and 13 mg/kg for intraperitoneal and subcutaneous administration, rats (Ostadalova, Babicky, 1980).

In whole facts of well-known metabolism, anti-carcinogenic, antiradical, immunomodulating properties and high biological activity indicate the prospects of Sec utilization for purposes of the human and livestock Se status optimization.

Synthesis of L-selenocystine (Sec-Sec) was achieved in the department of physics and chemistry of Penza State University of architecture and building (Patent No 2537166, RF)

Synthesis was achieved in two steps:

1) L-chloralanine was synthesized by modified method of Walsh (Walsh, 1971) from L-Ser:



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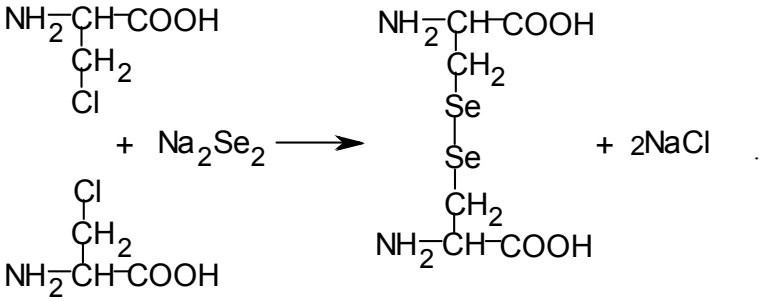
2) L-Sec-Sec was produced from L-chloralanine and sodium diselenide according to the patent data:



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L-Sec-Sec yield calculated on L-Ser was 30%. Reagents price for the laboratory synthesis was approximately $2 per 1 g.

Availability, stability, low toxicity and wide assortment of starting compounds (elemental Se, L-Ser), availability and relatively low reagents price, simplicity of allocation (filtration), high yields and stability of the resulting compound, simple apparatus utilized – all these properties indicate the prospects of industrial compound production.

The present work is devoted to the proof of Sec-Sec structure using capillary electrophoresis and estimation of potential possibility of its utilization as a source of Se in poultry on the background of mineral premix OMEK utilization (production of Biomed inc.).

MATERIALS AND METHODS

Analytical control of Sec-Sec structure was achieved using Sec-Sec samples of Sigma-Aldrich.

The analysis of Sec-Sec was achieved using capillary electrophoresis on Kapel 105M (Lumex) by M-04-38-2009 method.

Efficiency of Sec-Sec utilization as a feed additive in poultry was testified on broilers Cross 308 in the vivarium of Zagorsk technological Institute of aviculture (VNITIP) according to a scheme presented in Table 1, experimental feed recipes – according to recommendations of the Institute.

Chickens were grown in German cell battery "Big Dutchman", which includes systems of microclimate and poultry watering (35 chickens in each cell). Conditions of detention and feeding poultry were in accordance to VNITIP recommendations (Sies, 1993; Polyboyarinov et al., 2009). Distribution of feed was carried out manually.

Individual mass at the 7th, 14th, 21st and 33rd days of experiment, the safety of livestock, average daily weight gain, feed consumption value per 1 kg of mass were determined. Concentrations of Ca, P and trace elements in chicken bones were estimated

Raw ash in broilers' cnemis was determined by dry ashing at 450-500 ^{o}C. Ca, Zn, Mn, Fe and Se content in bones was estimated by AAS method on AA SPCTPA A "Duo 240FS/2407" spectrometer. Iodine concentration was determined colorimetrically by rodanonitrite method on KFK-photoelectrocolorimeter. Phosphorus levels in cnemis were indicated by photometric method according to VNITIP recommendations.

Table 1. ***Experiment scheme***

Group	Feeding characteristics
Control (group 1)	Feed with all-balanced nutrients (OBNF) with a premix of inorganic salts
Experimental (group 2)	OBNF with mineral premix OMEK 6% with 90 mg of OMEK 1; + sodium selenite (Se – 0.3 g/t of feed)
Experimental (group 3)	OBNF with mineral premix OMEK 6% with 90 mg of OMEK 1; + Sec-Sec (Se – 0.15 g/t of feed)
Experimental(group 4)	OBNF with mineral premix OMEK 6% with 90 mg of OMEK 1; + Sec-Sec (Se – 0.3 g/t of feed)

Table 2. ***Technical parameters of the experiment on broilers***

Indication	Group			
	Control	Na_2SeO_3 (Se – 0.3 g/t)	Sec-Sec (Se – 0.15 g/t)	Sec-Sec (Se – 0.3 g/t)
Broiler mass, g:				
at the age of	38.0	38.0	38.0	38.0
at the 7th day	139.04±1.92	142.76±2.26+2.68%	145.33±2.61+4.52%	144.77±2.30+4.07%
at 14th day	365.60±7.96	381.14±6.65+4.25%	367.90±5.65+0.63%	386.11±7.831+5.61%
at 21st day	676.69±15.04	689.60±9.53+1.91%	676.30±10.22–0.06%	708.43±15.9+4.69%
At 33 days, including:	1676.69±31.25	1690.06±21.83+0.8%	1660.57±24.03–0.92%	1699.31±34.5+1.35%
cocks	1785.5±55.78	1791.75±39.01+0.35%	1814.0±33.21+1.6%	1876.33±54.5+5.09%
hens	1619.91±32.51	1658.77±22.91+2.4%	1607.46±22.29–0.77%	1606.96±29.9–0.8%
Safety of livestock, %	100	100	100	100
Amount of feed per 1 broiler, kg	2.817	2.823	2.834	2.82
Amount of feed per 1 kg of mass increase, kg	1.719	1.708	1.747	1.697
Average daily gain, g	52.0	52.73	52.27	53.24

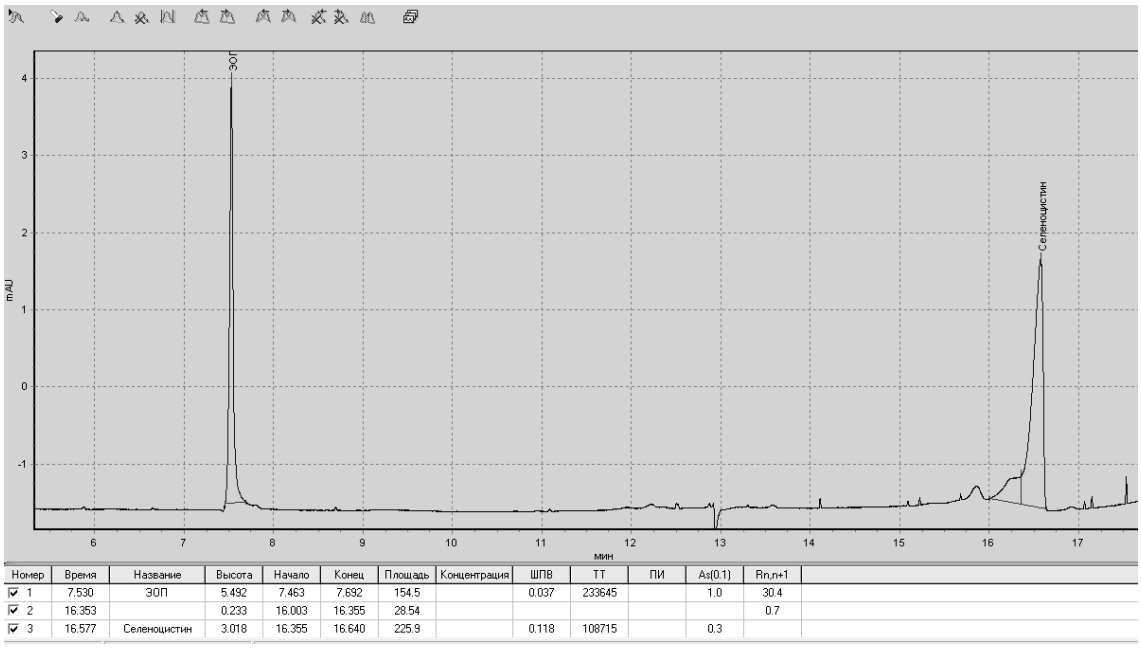
RESULTS

Electrophoregrams of synthesized Sec-Sec and Sec-Sec of Sigma-Aldrich are presented on Fig. 1, 2.

Time of electrophoretic flow both on the first and on the second electrophoregram was equal to 7.530 and 7.505 min accordingly. Time of Sec-Sec peak was at the 16.577 and 16.779 min indicating complete identity of both samples.

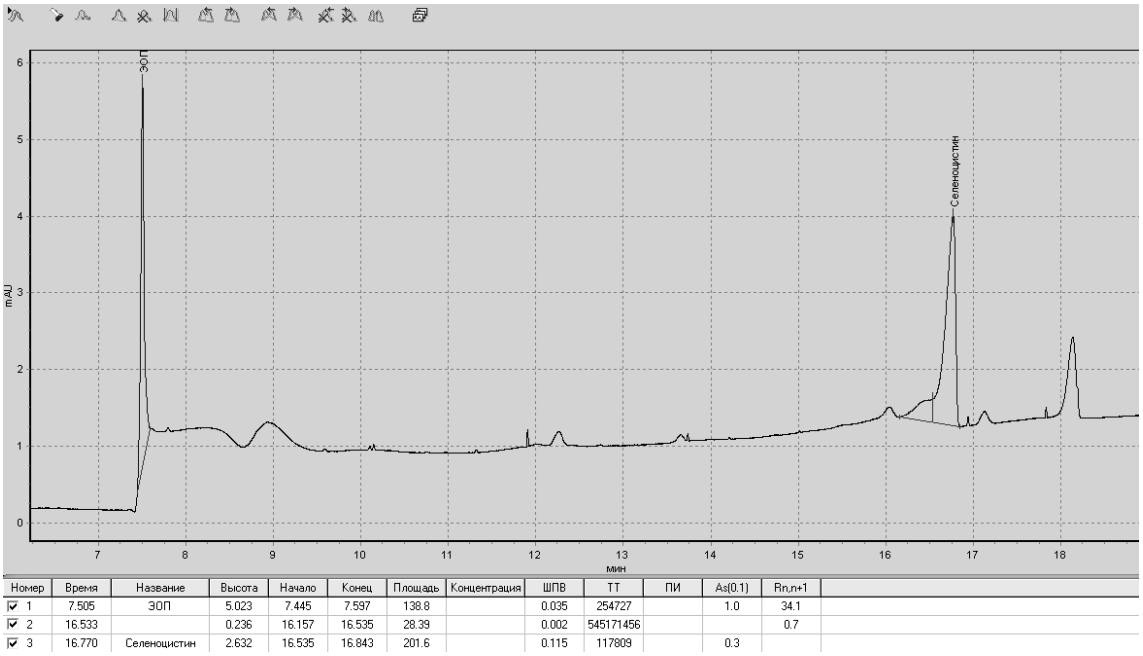
Results of Sec-Sec utilization efficiency in broiler feeding in shown in Table 2.

The results indicate that utilization of Sec-Sec in a dose of 0.3 g Se/t of feed in combination with mineral premix OMEK (based on aspartates of trace elements) in the 4th experimental group improves productive parameters of broilers compared to the control group (number 1) and to the second one where broilers received sodium selenite. Chicken mass in the 4th group by the end of fattening was enhanced by 1.35% compared to the control group accompanied by a 0.88 % decrease in feeding expenses per 1 kg of weight gain. Higher productivity in this group was revealed by us also in the first period of growth. Weight gain of broilers in this group was 4.69% higher than in the control group.



Номер	Время	Название	Высота	Начало	Конец	Площадь	Концентрация	ШПВ	ТТ	ПИ	As(0.1)	Rn,n+1
☑ 1	7.530	ЭОП	5.492	7.463	7.692	154.5		0.037	233645		1.0	30.4
☑ 2	16.353		0.233	16.003	16.355	28.54						0.7
☑ 3	16.577	Селеноцистин	3.018	16.355	16.640	225.9		0.118	108715		0.3	

Fig. 1. *Electrophoregram of synthesized Sec-Sec*



Номер	Время	Название	Высота	Начало	Конец	Площадь	Концентрация	ШПВ	ТТ	ПИ	As(0.1)	Rn,n+1
☑ 1	7.505	ЭОП	5.023	7.445	7.597	138.8		0.035	254727		1.0	34.1
☑ 2	16.533		0.236	16.157	16.535	28.39		0.002	545171456			0.7
☑ 3	16.770	Селеноцистин	2.632	16.535	16.843	201.6		0.115	117809		0.3	

Fig. 2. *Electrophoregram of Sigma-Aldrich Sec-Sec*

Utilization of Sec-Sec in a dose of 0.15 g Se/t happened to be less efficient than sodium selenite (group No 2).

Deterioration of feed conversion by 1.63 % and higher feed expenses per 1 broiler in this group indicate that further decrease of Sec-Sec dose may become ineffective. The results show that supplementation of broilers with Sec-Sec should be achieved in a dose not less than 0.15–0.3 g Se/t.

Ca, P and trace elements content in cnemis of broilers is presented in Table 3.

As it can be seen from Table 3, Se concentrations in broilers' bones of the 2nd and the 4th group were identical and exceeded control data by 0.07 mg/kg. Lower Sec-Sec doses in the 3d group resulted in a decrease of bone Se concentration by 0.04 mg/kg compared to the 4th group data but still exceeded values of the control group by 0.03 mg/kg.

Table 3. ***Content of Ca, P, trace elements (mg/100 g) and amount of ash (%) in broilers' bones at the age of 34 days***

Parameters	Group			
	Control	Na_2SeO_3 (Se – 0.3 g/t)	Sec-Sec (Se – 0.15 g/t)	Sec-Sec (Se – 0.3 g/t)
Ash	46.23±3,70	47.71±3,34	48.47±0,97	51.37±2,57
Ca	18.50±1,29	18.50±0,92	17.94±1,44	19.44±0,97
P	7.97±0,80	8.30±0,08	8.23±0,17	8.38±0,33
Fe	20.50±1,43	23.72±0,95	19.02±0,92	18.00±1,75
Mn	0.698±0,07	0.657±0,033	0.510±0,05	0.569±0,04
Zn	15.54±0,46	14.11±0,56	12.71±0,64	16.05±1,44
Cu	0.144±0,008	0.232±0,007	0.141±0,05	0.228±0,016
Se, mg/kg	0.17±0,012	0.24±0,01	0.20±0,01	0.24±0,015
I	1.27±0,039	1.89±0,076	2.42±0,12	2.07±0,19

In a whole Sec-Sec shows the highest and most stable stimulating effect in broilers feeding, Besides this Sec-Sec utilization (0.3 g Se/t) demonstrates the highest average daily weight gain (53.24 g/day) and a decrease in feed expenses per 1 kg of weight gain (1.697 kg) compared to the control broilers (52.0 g/day and 1.719 kg accordingly).

CONCLUSIONS

The results prove the identity of synthesized Sec-Sec and Sec-Sec of Sigma Aldrich, and suggest the efficiency of Sec-Sec use in broilers feeding in a dose of 0.15–0.3 g Se/t.

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СИНТЕЗ СЕЛЕНОЦИСТЕИНА И ПЕРСПЕКТИВЫ ЕГО ПРИМЕНЕНИЯ ДЛЯ КОРРЕКЦИИ СЕЛЕНОДЕФИЦИТА

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РЕЗЮМЕ. Широко применяемые для коррекции селенового дефицита животных и человека неорганические соединения селена, обогащенные селеном дрожжи и селенсодержащие ксенобиотики, обладают рядом существенных недостатков. Аминокислота L-селеноцистеин (окисленная форма – L-селеноцистин), обладая полной физиологической совместимостью, наличием антиканцерогенной, антирадикальной, иммуномодулирующей и протекторной функциями является перспективным соединением как в плане коррекции селенового дефицита животных и человека, так и биологической активности.

На кафедре «Физики и химии» Пензенского ГУАС был разработан и проведен синтез аминокислоты L-селеноцистина, получен патент № 2537166 (РФ). Аналитическое сравнение L-селеноцистина методом капиллярного электрофореза на приборе «Капель 105 М» показали идентичность образцов синтезированной аминокислоты и фирмы «Sigma Aldrich».

Для определения потенциальных возможностей L-селеноцистина на фоне минерального премикса ОМЭК (L-аспарагинаты микроэлементов) был заложен опыт на цыплятах-бройлерах кросса «Ross 308». Применение селеноцистина в дозе 0,3 г/т корма (в расчете на чистый элемент селен) позволило увеличить прирост живой биомассы птицы на 1,35% при снижении на 0,88% затрат корма, по сравнению с контрольной группой и группой в корм которой добавляли селенит натрия (Se – 0,3 г/т). Анализ содержания микроэлементов большеберцовой кости показал, что при использовании L-селеноцистина в дозе 0,3 г/т содержание селена в костяке бройлеров составило 0,24 мг/кг, как у группы которая получала селенит натрия (Se – 0,3 г/т). Это превышает содержание селена на 0,07 мг/кг в контрольной группе.

КЛЮЧЕВЫЕ СЛОВА: селен, селеноцистин, синтез, капиллярный электрофорез, бройлер, ростостимулирующий эффект, прирост живой массы.