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## ANIMAL HUSBANDRY

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### APPLICATION OF A NATURAL ANTIOXIDANT FOR PROTECT FEED VITAMIN A

Research article

#### Abstract

The article discusses aspects that affect the stability and bioavailability of feed vitamin A, as well as the importance of using antioxidants to protect feed vitamin A during storage. The aim of the study was to study the effect of antioxidants from the chaga mushroom on the stability and bioavailability of feed vitamin A. Methods for determining the bioavailability in vitro in biorelevant dissolution media were used in the study. It has been found that the introduction of the mushroom extract made it possible to increase the bioavailability in the stomach from 5.53 to 8.61%. Introduction of the mushroom extract made it possible to increase the bioavailability of the feed vitamin from 1.7 to 2.64%. Thus, it was shown that the introduction of the extract contributes to an increase in the bioavailability of microencapsulated vitamin A to a greater extent in the stomach than in the intestine. High solubility of experimental samples 2 and 3 was noted in the media of the stomach and intestines, respectively. The scientific novelty of the work lies in the establishment of the role of the natural antioxidant of the chaga mushroom as a component of the food matrix, contributing to an increase in the bioavailability of the fat-soluble vitamin. Thus, it has been shown that the introduction of the extract allows to increase the bioavailability of microencapsulated feed vitamin A.

**Keywords:** retinol acetate, FaSSIF, FaSSGF, nutritional matrix, biorelevant dissolution media.

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### ПРИМЕНЕНИЕ НАТУРАЛЬНОГО АНТИОКСИДАНТА ДЛЯ ЗАЩИТЫ КОРМОВОГО ВИТАМИНА А

Научная статья

#### Аннотация

В статье рассмотрены аспекты, влияющие на стабильность и биодоступность кормового витамина А, а также важность использования антиоксидантов для защиты кормового витамина А при хранении. Целью исследования было изучение влияния антиоксидантов гриба чага на стабильность и биодоступность кормового витамина А. В исследовании использовались методы определения биодоступности in vitro с использованием биорелевантных сред. Установлено, что введение экстракта гриба позволило увеличить биодоступность в желудке с 5,53 до 8,61%. Введение экстракта гриба позволило повысить биодоступность кормового витамина с 1,7 до 2,64%. Таким образом, показано, что введение экстракта способствует повышению биодоступности микрокапсулированного витамина А в большей степени в желудке, чем в кишечнике. Отмечена высокая растворимость опытных образцов 2 и 3 в средах желудка и кишечника соответственно. Научная новизна работы заключается в установлении роли антиоксидантов гриба чаги как компонентов пищевой матрицы, способствующих повышению биодоступности жирорастворимого витамина. Таким образом, показано, что введение экстракта позволяет повысить биодоступность микрокапсулированного кормового витамина А.

**Ключевые слова:** ретинола ацетат, FaSSIF, FaSSGF, пищевая матрица, биорелевантный растворитель.

## 1. Introduction

One of the most important factors that determine the level of productivity of farm animals, the quality of livestock products is feeding, the provision of the diet with vital nutrients. Vitamin A participates in the regulation of the metabolism of fats, carbohydrates and proteins, and also has a positive effect on fertility, which helps to increase the body's resistance to diseases and has an important role in the renewal and development of the skin and protection of membranes, as well as ensuring the normal functioning of the visual process.

Bioavailability is the amount of nutrients released from the food matrix before being absorbed from the small intestine [3, P. 1345].

There is evidence that the bioavailability of lipophilic bioactive substances, in particular vitamin A, varies from 10 to 80%, depending on the nature of the food matrix [6, P. 3-4], [7, P. 4640], [9, P. 265-267], [10, P. 129-148]. This relationship underscores the importance of developing and studying the components of nutritional matrices to increase the bioavailability of vitamins.

Components present in feed, including proteins, polysaccharides, minerals, and other additives, can also affect the bioavailability of vitamins, disrupting lipid digestion and absorption [2, P. 954-960].

The stability of vitamin A in fortified feeds can be improved through a variety of strategies: managing storage factors and packaging conditions (e.g. temperature, light, oxygen and humidity) [4, P. 90-98], controlling food matrix conditions (e.g. pH and water purity), adding antioxidants or chelating agents. The bioavailability of vitamin A depends on their behavior. In the human intestine after ingestion, which is considered the remainder of this section. Vitamin A in fortified foods is usually dissolved in oil. As a result, absorption of encapsulated vitamin A in the gastrointestinal tract (GIT) strongly correlates with lipid digestion. Also, the bioavailability of vitamin A in different food matrices can vary significantly depending on how it reacts to the chain of events that occur after ingestion: various physical, chemical (changes in pH, changes in composition) and biochemical (enzyme activity) occurring during digestion in the oral cavity, stomach, small intestine.

Structurally, retinol forms a beta-ionone ring attached to an isoprenoid chain, which is also called a retinyl group, whereas  $\beta$ -carotene is made up of two retinyl groups linked through their isoprenoid chains. The structure of retinyl plays a vital role in the activity of vitamin A, as it contains an electron-dense region that can bind active substances such as free radicals, thereby reducing oxidative stress [8, P. 469-476]. The high chemical reactivity of vitamin A means that it is susceptible to oxidative degradation and/or isomerization, especially when exposed to light, heat, metals and oxidants [1, P. 125-126]. The four-side chain double bonds, which allow 16 different retinol configurations, along with the hydroxyl group at the fifteenth position of the carbon chain, are responsible for the poor overall stability of vitamin A. The hydroxyl group and the four double bonds are easily oxidized so that light, trace elements and heat can cause the vitamin to decompose. Due to the inability to modify the side chain containing the double bonds that would lead to a reduction in biological activity. Using some organic acids (acetic, propionic and palmitic) the free hydroxyl group can be esterified, thus forming retinol acetate, retinol propionate and retinol palmitate. Although these three compounds differ significantly in melting point, UV absorption and fluorescence, they have the same stability. Retinol acetate is used in the feed industry, retinol propionate is used for liquid applications due to its low melting point, and retinol palmitate is often used as an ingredient in food because it is more chemically stable than free retinol. Despite this, it is necessary to use methods to protect vitamin A from degradation during production and storage processes.

As an antioxidant protection of feed and premixes for animals and poultry, the synthetic antioxidant ethoxyquin is widely used, which prevents fat oxidation and neutralizes toxic substances formed during lipid oxidation. In the European Union (EU) ethoxyquin (E324) has been approved as a feed additive (antioxidant) since 1970 for all animal species with a restriction on the amount. The upper limit for ethoxyquin and other antioxidants (BHA and BHT) is approximately 150 mg per kg complete animal feed. However, the European Food Safety Authority (EFSA) has discovered that one of its metabolites, ethoxyquinone imine, may be genotoxic, meaning it can damage DNA, indicating a potential safety hazard. As a result of the production process, this feed additive contains an admixture of p-phenetidine, which is a possible mutagen. Animal studies have shown that in some cases, minimum levels of ethoxyquin and its metabolites are transferred from feed to animal products.

An alternative is the use of natural antioxidants to increase the chemical stability of vitamin A. One of the sources of highly active antioxidant compounds is the fungus *Inonotus obliquus* (Ach. Pers.) Pilát (1942). Extracts of the chaga mushroom *Inonotus obliquus* (Ach. Pers.) Pilát (1942) contain a high molecular weight pigment or polyphenolic complex with a complex chemical composition. Compounds of the polyphenolic complex of the fungus *Inonotus obliquus* (Ach. Pers.) Pilát (1942) have biologically active properties: antibacterial, antitumor, anti-inflammatory, antioxidant, and effectively suppress the peroxidation of polyunsaturated fatty acids. The main substance of the chaga mushroom *Inonotus obliquus* (Ach. Pers.) Pilát (1942), which has high antioxidant and antiradical activity, is melanin. Many authors explain the antioxidant properties of mushroom extracts by the high content of polyphenolic compounds such as lanosterol, inotodiol, trametenolic acids, and ergosterol peroxides [5, P. 200].

## 2. Methods

The aim of the study was to study the effect of antioxidants from the chaga mushroom on the stability and bioavailability of feed vitamin A. For this, the following tasks were set: to determine the bioavailability of experimental samples of microencapsulated vitamin A in biorelevant media of the stomach and intestines; to determine the bioavailability of experimental samples of microencapsulated vitamin A in biorelevant media of the stomach and intestines with the antioxidant of the chaga mushroom.

Biorelevant media "(biorelevant media), allowing to simulate the behavior, dissolution and absorption of the investigated substance in the gastrointestinal tract. The use of biorelevant media makes it possible to predict the influence of the composition of excipients and the effects of food more accurately on the solubility and bioavailability of drugs for internal application. Biorelevant dissolution media simulate gastrointestinal were used FaSSIF (Fasted-state simulated intestinal fluid),

pH 6,5, FaSSGF (Fasted-State Simulated Gastric Fluid), pH 1,6 (Biorelevant, United Kingdom). Experimental samples of microencapsulated vitamin A (retinol acetate) (1,000,000 IU/g) produced by LLC «Arnika» (ES1, ES2, ES3). A lyophilized extract of the chaga mushroom *Inonotus obliquus* (Ach. Pers.) Pilát (1942) obtained using supercritical fluid extraction with carbon dioxide was used as a natural antioxidant; it has an antiradical activity equivalent to that of ascorbic acid at a concentration of 394.54 µg/ml. Samples of microencapsulated vitamin A with an activity of 15,000 IU (5.16 mg of retinol acetate) were added to 10 ml of biorelevant medium. In the test samples with the extract, 50 mg of the extract of the chaga mushroom *Inonotus obliquus* (Ach. Pers.) Pilát (1942) were added. The content of vitamin A (retinol acetate) was determined by HPLC on a Shimadzu LC-20 Prominence HPLC chromatograph (Shimadzu, Japan) equipped with an SPD-20A UV-VIS detector (Shimadzu, Japan) and a Shodex Asahipak C8P-50G 4A reverse phase column (4.6 × 10 mm, particle size: 5 µm; Shodex, Japan).

### 3. Results

For several drugs and vitamins in classical pharmacopoeial buffer solutions, their dissolution under in vivo conditions is not reflected with a sufficient degree of reliability. To solve this problem, biorelevant media were used to simulate the behavior, dissolution, and absorption of the drug in the gastrointestinal tract.

As a result of an experiment to study the bioavailability of feed vitamin A in the stomach (FaSSGF), it was found that among all tested samples of microencapsulated vitamin A, the highest bioavailability is characterized by a sample of vitamin ES2 - 34.88%. The introduction of the mushroom extract made it possible to increase the bioavailability in the stomach from 5,53 to 8,61% (Figure 1).

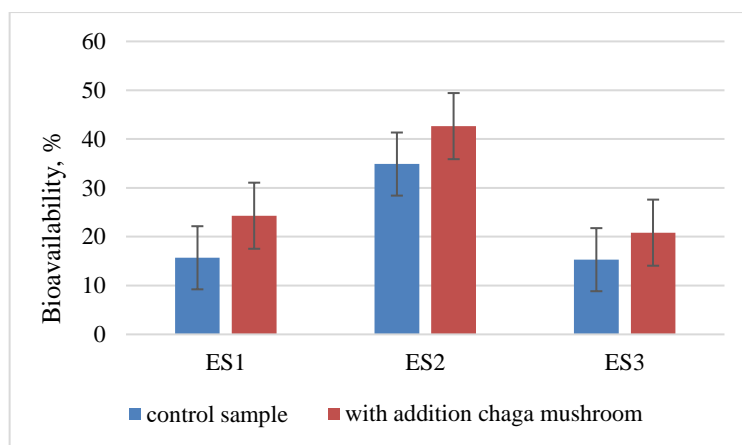


Fig. 1 – Bioavailability of feed vitamin A in the stomach (FaSSGF)

The results of dissolution of samples of vitamin A in the intestinal environment (Figure 2) indicate lower bioavailability values compared to data on bioavailability in the stomach. However, the greatest release in the intestinal environment was noted for experimental sample 3, which is 9.88%. The introduction of the mushroom extract made it possible to increase the bioavailability of the feed vitamin from 1,7 to 2,64%.

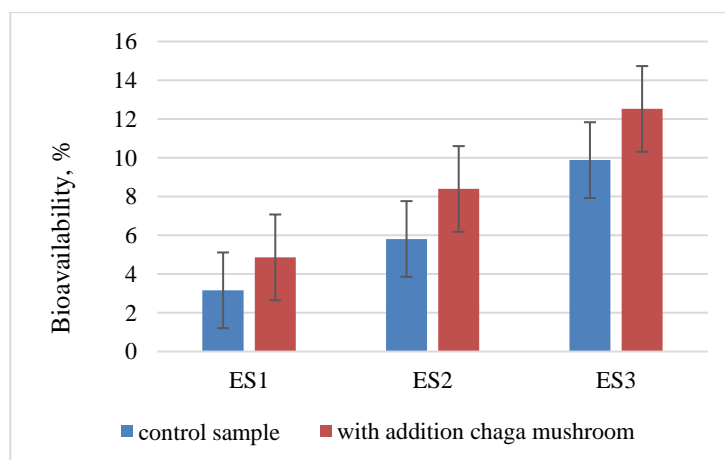


Fig. 2 – Bioavailability of feed vitamin A in the intestine (FaSSIF)

Thus, it was shown that the introduction of the extract contributes to an increase in the bioavailability of microencapsulated vitamin A to a greater extent in the stomach than in the intestine. High solubility of experimental samples 2 and 3 was noted in the media of the stomach and intestines, respectively.

The established effect of increasing bioavailability can be associated with the action of a high molecular weight polyphenolic complex of antioxidants of the chaga mushroom [5, P. 198-202]. Biologically active compounds of the chaga mushroom act as natural antioxidants. Extracts of the chaga mushroom contain a high molecular weight pigment or

polyphenolic complex containing melanins. Some authors point to the influence of components of food systems, including antioxidants, on the processes of digestion and absorption of vitamin A [7, P. 4642-4645], [9, P. 265-270]. In this regard, the introduction of an antioxidant helps to increase the bioavailability of vitamin A in the stomach and intestine.

This article emphasizes the importance of antioxidant compounds in the nutritional matrix for the stability and bioavailability of feed vitamin A. The stability of feed vitamin A can be increased by using natural antioxidant compounds, in particular the antioxidants of the chaga mushroom *Inonotus obliquus* (Ach. Pers.) Pilát (1942). It has been shown that the addition of the extract as an auxiliary substance for the antioxidant protection of vitamin A has no effect on the release of the active substance in the stomach and intestines.

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#### Conflict of Interest

#### Конфликт интересов

None declared.

Не указан.

#### References

1. Carlotti M.E. Photostability and Stability over Time of Retinyl Palmitate in an O/W Emulsion and in SLN Introduced in the Emulsion / M.E. Carlotti, S. Sapino, M. Trotta et al. // Journal of Dispersion Science and Technology. – 2005. – V. 26. – №2. – P. 125-138. – DOI: 10.1081/DIS-200045403
2. Dima C. Bioavailability of nutraceuticals: Role of the food matrix, processing conditions, the gastrointestinal tract, and nanodelivery systems / C. Dima, E. Assadpour, S. Dima et al. // Comprehensive Reviews in Food Science and Food Safety. – 2020. – V. 19 (3). – P. 954-994. – DOI: 10.1111/1541-4337.12547
3. Heaney R.P. Factors influencing the measurement of bioavailability, taking calcium as a model / R.P. Heaney // J Nutr. – 2001. – V. 131. – № 4. – P. 1344S–1348S. – DOI: 10.1093/jn/131.4.1344S.
4. Hemery Y.M. Influence of light exposure and oxidative status on the stability of vitamins A and D (3) during the storage of fortified soybean oil / Y.M. Hemery, L. Fontan, R. Moench-Pfanner et al. // Food Chemistry. – 2015. – V. 184. – P. 90-98. – DOI: 10.1016/j.foodchem.2015.03.096
5. Hye-Kyung Se. Antioxidant Activity of Subcritical Water Extracts from Chaga Mushroom (*Inonotus obliquus*) / Se Hye-Kyung // Separation Science and Technology. – 2001. – V. 45. – № 2. – P. 198-203.
6. Reboul E. Vitamin E Bioavailability: Mechanisms of Intestinal Absorption in the Spotlight / E. Reboul // Antioxidants (Basel). – 2017. – V. 6 (4). – DOI:10.3390/antiox6040095
7. Salvia-Trujillo L. Improvement of  $\beta$ -carotene bioaccessibility from dietary supplements by addition of excipient nanoemulsions / L. Salvia-Trujillo, S.H. Verkempinck, L. Sun et al. // Journal of Agricultural and Food Chemistry. – 2017. – V. 64. – №2. – P. 4639-4647.
8. Sauvant P. Vitamin A enrichment: Caution with encapsulation strategies used for food applications / P. Sauvant, M. Cansell, A. Hadj Sassi et al. // Food Research International. – 2012. – V. 46. – № 2. – P. 469–479.
9. Tan Y. Impact of an indigestible oil phase (mineral oil) on the bioaccessibility of vitamin D3 encapsulated in whey protein-stabilized nanoemulsions / Y. Tan, J. Liu, H. Zhou et al. // Food Research International. – 2019. – V. 120. – P. 264-274.
10. Tan Y., David J. Improving the bioavailability of oil-soluble vitamins by optimizing food matrix effects: A review / Y. Tan, J. David // Food Chemistry – V. 348. – 2021. – P. 129-148. – DOI: 10.1016/j.foodchem.2021.129148.