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В матеріалах статті наведено роль та призначення ферментів у кормовиробництві, зокрема для птахівництва. Надано характеристику складу мультиферментного препарату Ветозим Мульти компанії «Ветсинтез», який містить комплекс ферментативних активностей: глюканазу, ксиланазу, целюлазу, протеазу, амілазу, маннаназу, фітазу. Представлені практичні результати використання мультиферментного препарату Ветозим Мульти у складі комбікормів для бройлерів. Розраховано рецепти комбікорму гровер для бройлерів та встановлено оптимальну норму введення ферменту у кількості 100 г на 1 т. Проведено порівняння відсотку введення мікрокомпонентів до складу комбікорму гровер для бройлерів та розраховано показники поживності комбікорму без та з використанням мультиферментного препарату Ветозим Мульти. Встановлено, що введення ферменту дозволяє зменшити в складі рецепта від 20 до 30 % монокальцій фосфат, за рахунок наявної фітазної активності. Ветозим Мульти значно підвищує ефективність використання корму, спостерігається збільшення середньодобових приростів маси бройлерів на 1..2,5 г, зниження показника конверсії комбікорму для птиці на 0,02...0,07 та зниження собівартості рецептів в межах 300-500 грн/т, що стало можливим завдяки оптимізації використання продуктів переробки соняшнику та зернових з високим вмістом клітковини. Наявність у складі препарату Ветозим Мульти термостабільних ферментів дозволяє використовувати його при виробництві комбікормів з подлибленою тепловою обробкою при температурі кондиціювання та гранулювання до +90<sup>o</sup>C, що сприятиме більш повному розщепленню та засвоєнню сировинних компонентів, зокрема вуглеводів, білків та клітковини. Розрахунок економічних показників свідчить, що препарат Ветозим Мульти не тільки забезпечує збалансоване харчування для птиці, але й дозволяє знижувати вартість кормів за рахунок ефективнішого використання менш дорогих компонентів.

**Ключові слова:** ферменти, активність, препарат Ветозим Мульти, корми, ефективність, птахівництво.

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**FEED LYSIN. MEANING AND SYNTHESIS****Abstract**

The intensification of animal husbandry depends on the full feeding of farm animals, which provides for the content of the most important nutrients and biologically active substances in the feed. In order to obtain the necessary amount of high-quality livestock products, to ensure high reproductive capacity of animals, specialists develop animal feeding rations taking into account minimum feed costs. Providing farm animals with fodder protein is one of the important tasks of agriculture and enterprises that produce protein fodder or obtain it in the form of by-products. The use of by-products of food processing is of great importance in animal feeding programs and also solves the problem of disposal of these by-products. The application of such solutions will allow to reduce the need of farms for fodder grain, fodder of animal origin and other expensive components used in feeding. Protein feeds of plant origin include sunflower processing products as the cheapest source of feed protein in Ukraine. The inferiority of the proteins of sunflower cake and meal should be compensated with synthetic amino acids. Lysine, as an essential amino acid, has a wide range of applications, covering various fields from medicine to the food industry and agricultural activities, in particular in the feed industry. Most of the produced lysine is used in the production of feed additives (for pigs and poultry), which significantly increase the nutritional value of vegetable feed with a low lysine content. The addition of lysine to compound feed improves protein digestibility by animals and reduces feed costs for the production of livestock products. Given the availability of lysine and taking into account livestock statistics, namely the increase in the number of pigs and the decrease in the number of cattle and poultry, industrial production of lysine as a feed additive for pigs is a promising direction. The main methods of lysine production are considered - chemical, chemical-enzymatic, biological and microbiological. The technology of each method is analyzed in detail, the advantages and disadvantages of different methods of obtaining lysine are given.

**Keywords:** animal husbandry, protein, protein feed, amino acids, lysine, production methods, technology.



**Introduction. Formulation of the problem**

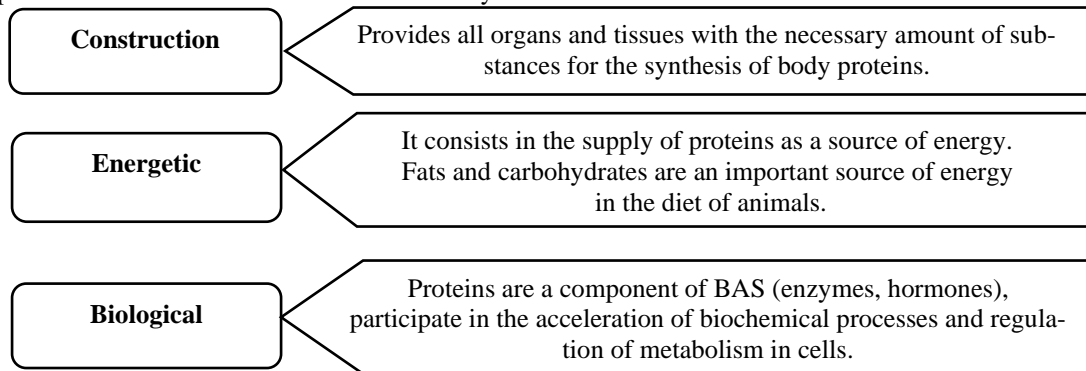
For the full development of agricultural animals and poultry, high-quality feed is necessary, which is able to satisfy the needs of the animal body in nutrients. In the system of comprehensive assessment of feed nutrition, a special role is played by protein, which performs a number of functions, primarily structural, energetic and biological, in ensuring the vital activity of the animal organism (Fig. 1). [1]

Protein is part of biologically active substances and plays an important role in the vital activity of organisms (reproduction, development, productivity, etc.). The role of protein in the diets of farm animals is very im-

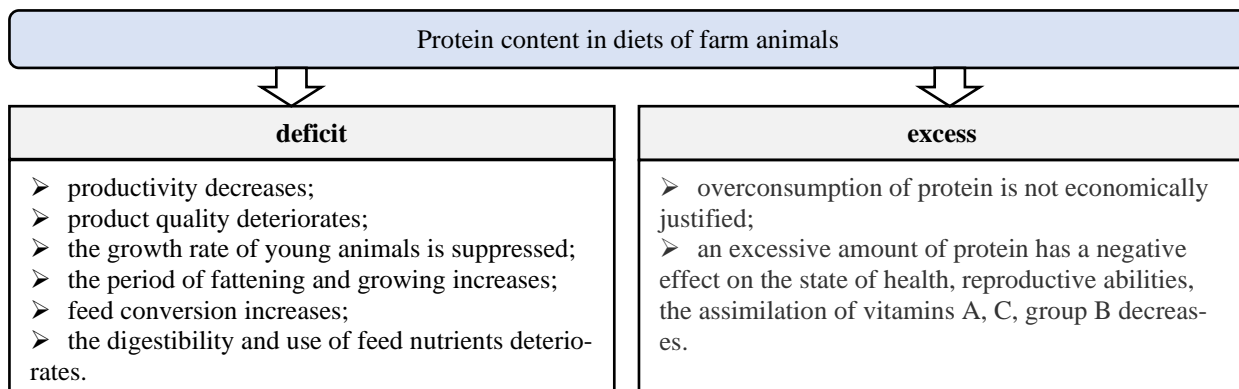
portant and is determined by the amino acid composition, essential amino acids. Because insufficient and excess protein can lead to negative consequences (Fig. 2). [1].

Balancing the diet in terms of protein content is carried out by using protein feeds of vegetable and animal origin, as well as feed additives. The protein content in feed of animal origin is almost 2 times greater than in feed of plant origin, and the content of digestible protein in feed of animal origin is 1.2-1.5 times greater than in feed of plant origin (Table 1). [2-4].

Animal feed proteins have a much higher content of essential amino acids - lysine and methionine.



**Fig. 1. The main functions of protein**



**Fig. 2. Influence of protein content in diets of farm animals**

**Table 1 – Amino acid composition of animal and vegetable protein feeds**

Indicators	Fodder of animal origin			Fodder of plant origin			
	Meat and bone meal	Meat meal	Fish meal	Sunflower cake	Soy cake	Sunflower meal	Soy meal
Content, %							
dry matter	86.5	90.0	88.0	89.2	90.0	90.0	89.1
protein	41.8	56.1	59.1	33.4	41.8	34.0	37.2
Content in 1 kg:							
digestible protein, g	344	516.0	532.0	307.0	393.0	386	388
Amino acids, g:							
Lysine	35.3	40.4	47.1	13.1	26.3	14.2	28.2
Methionine + cystine	11.4	12.9	25.5	15.4	11.3	16.7	10.8
Tryptophan	5.1	5.8	5.3	6.0	5.7	9.9	4.8
Macronutrients, g:							
Calcium	94.9	61.0	59.3	3.3	4.3	3.6	2.6
Phosphorus	47.8	31.0	32.6	11.6	6.9	6.7	6.6
Magnesium	1.9	0.9	4.53	5.4	2.9	5.1	3.3
Potassium	2.8	5.8	16.6	10.4	17.4	8.0	19.5
Sodium	8.7	10.1	16.9	0.6	0.5	0.4	1.9
Chlorine	4.3	8.1	10.6	1.0	0.9	0.4	0.4



Among plant-based feeds, the by-products of soy processing contain the same amount of protein as the amount of protein in animal feeds, but the amino acid composition of soybean cake and soybean meal is significantly inferior to animal proteins, especially lysine and methionine. The cost of by-products of soybean processing is much higher than plant protein sources. In addition, the use of soy and by-products of its processing in feed limits the presence of anti-nutrients that cause digestive disorders and reduced performance. Given this fact, soybeans require mandatory heat treatment so that they can be used as feed for farm animals. Monitoring the activity of anti-nutritive properties of soybeans is complicated and not always effective, and the cost of heat treatment is significant. This limits the use of soy protein in feeding animals and poultry.

Protein feeds of plant origin include sunflower processing products as the cheapest source of feed protein in Ukraine. [5]

The inferiority of the proteins of sunflower cake and meal should be compensated with synthetic amino acids. The use of synthetic amino acids makes it possible to rationally balance the composition of compound feed with essential amino acids. This is significantly cheaper than using natural sources, although it requires a more subtle approach to balancing problems.

Lysine, as an indispensable amino acid, has a wide range of applications, covering various fields from medicine to the food industry and agricultural activities, in particular in the feed industry. The addition of lysine to compound feed improves protein assimilation by animals and reduces feed costs for the production of livestock products.

The synthesis of lysine began its history with the study of amino acids at the end of the 19th century. Lysine was discovered by the German chemist Heinrich Dresel in 1889 as part of casein. Later, thanks to the development of technologies, the synthesis of lysine as a separate molecule became possible, which made its production economically profitable. [6-9]

It is necessary to satisfy the needs of the animal in amino acids, so that they enter the feed in the form of protein or in the form of crystals. The required amount depends on the species and age of the animal. The order of limiting amino acids can be determined by comparing the actual amino acid requirements of the feed. Limiting amino acids for pig and broiler diets consisting of corn (or grain) and soybean meal are shown in Table 2.

Crystalline amino acids must be added to feed when the amount of protein contained in them decreases. This fact is the main reason for the use of methionine and lysine hydrochloride in feed. The use of a more economical source of threonine and tryptophan has entered a new era in which the barriers of the 2nd and 3rd limiting amino acids have been overcome.

**Table 2 - Sequence of limiting amino acids**

	First	Second	Third
Broiler	Methionine	Lysine	Threonine
A pig in the growth phase	Lysine	Threonine	Tryptophan

A lack of lysine leads to a decrease in appetite, weight loss, a decrease in the laying capacity of birds, a violation of bone tissue calcification, general exhaustion and the development of anemia in the event of a violation of hematopoiesis and hemoglobin synthesis. With a lack of lysine in the diet and a sufficient amount of amino acids, paralysis and depigmentation of poultry plumage occur. Lysine is extremely necessary for poultry to regulate the exchange of nitrogen and carbohydrates, as well as for the synthesis of the most important proteins - nucleoproteins, chromoproteins, etc. [7-8].

**The purpose of the study**

The purpose of the work is to analyze and characterize the methods of production of fodder lysine for obtaining high-quality livestock products.

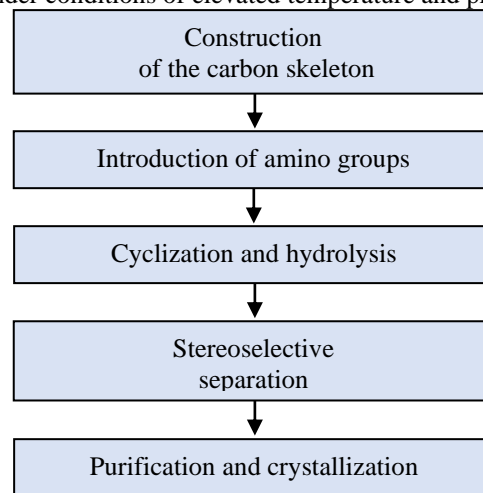
The production of lysine is based on four main methods: chemical, chemical-enzymatic, microbiological and protein hydrolysis. Each method has its own characteristics, advantages and disadvantages and requires specific equipment.

*Chemical method of obtaining lysine*

The chemical method of obtaining lysine is a multistage process based on the sequential transformation of simple organic compounds into the target product using organic reactions: amination, separation of isomers, cyclization, hydrogenation (Fig. 3).

The first stage of synthesis is the construction of the carbon skeleton of lysine; for this, hydrocarbon compounds containing the required number of carbon atoms are used. The step includes condensation, rearrangement/addition reactions. First, aldehydes/ketones condense with nucleophiles to form new bonds, then a rearrangement step (Claisen reaction) takes place to lengthen the chain, and functional groups are added via Michael addition (nucleophilic addition). The process is completed by hydrogenation to form amino acid groups, creating the complete carbon skeleton of lysine.

The second stage is the introduction of amino groups (NH<sub>2</sub>) into the α- and ε-positions of the carbon chain. The process is carried out using reactions of nucleophilic substitution or reduction of nitro compounds. The hydroamination reaction catalyzed by transition metal compounds allows the efficient introduction of the first amino group, forming ε-aminocaproic acid. The second amino group is introduced by reaction with α-ketoacids under conditions of elevated temperature and pres-



**Fig. 3. The main stages of the chemical synthesis**

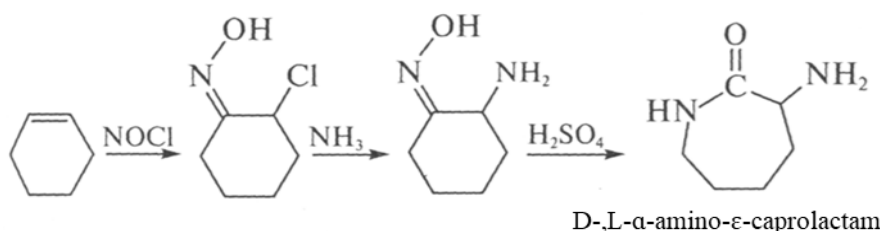


Fig. 4. Lactam hydrolysis of lysine

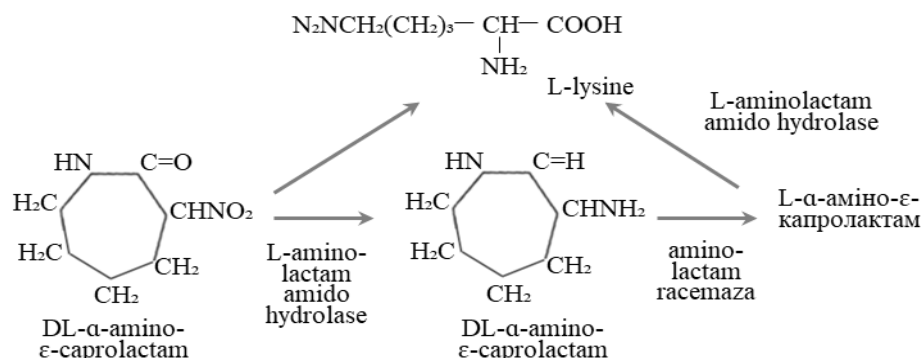


Fig. 5. Stereoselective separation of the racemate

sure, which promotes the nucleophilic attack of ammonia or hydrazine on the carbonyl group. The obtained compound undergoes cyclization with the formation of a lactam, which is a thermodynamically advantageous process. The next stage – lactam hydrolysis – allows obtaining the target compound –  $\alpha,\epsilon$ -diaminocaproic acid (lysine) (Fig. 4).

Since chemical synthesis, as a rule, leads to the formation of a racemic mixture of L- and D-isomers of lysine, a step of stereoselective separation is necessary (Fig. 5).

For this, ion exchange chromatography methods are widely used. An alternative method is the use of enzymes that selectively interact with one of the isomers.

Purification of the obtained L-lysine is carried out by means of recrystallization from appropriate solvents - ethanol or water, which allows removing impurities and obtaining a pure product with a high degree of purity, suitable for further use.

For the chemical synthesis of lysine, specific equipment is used, including high-pressure autoclaves made of stainless steel with a coating resistant to ammonia and high temperatures; reactors equipped with catalysts based on nickel or platinum; ion exchange columns for separation of isomers; chromatographic installations; vacuum drying systems.

Well-known companies that use the chemical method in their processes for the production of lysine are Ajinomoto Co. Inc. та Evonik Industries.

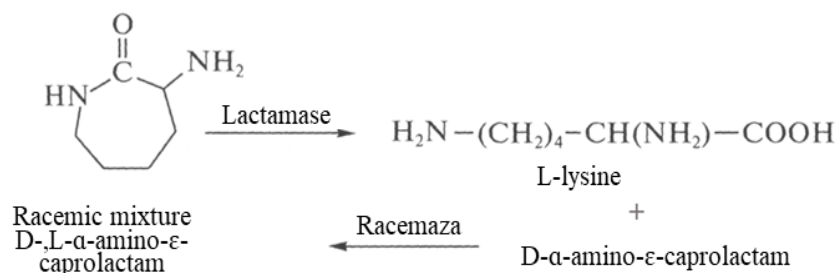


Fig. 6. Obtaining a racemic mixture of caprolactam

Independence from biological systems and the possibility of precise control over the process are undeniable advantages of the chemical synthesis of lysine. However, the high cost associated with the use of complex equipment and the need to dispose of by-products is a significant limitation of this method.

However, the high cost associated with the use of complex equipment and the need to dispose of by-products is a significant limitation of this method. It is worth noting that chemical synthesis played a pioneering role in the development of industrial production of amino acids, which laid a certain foundation for further research in biotechnology.

In the conditions of the modern feed additives market, the main criterion is economic efficiency and environmental safety, which chemical synthesis is unable to provide. [10]

#### Chemical-enzymatic method of obtaining lysine

The chemical-enzymatic method of lysine production has become widespread in Japan and the USA. This method was proposed by the company "ToyoRayon" ("Torai") in the 70s of the last century. This approach makes it possible to create a highly efficient technology based on a combination of chemical synthesis of an intermediate product ( $\alpha$ -amino- $\epsilon$ -capro-lactam) and its subsequent enzymatic conversion into lysine.

First, the synthesis of "precursors" - the corresponding carboxylic acid - is carried out using a chemical method, then the acid is converted into the corresponding amino acid. Such bioconversion is carried out by enzymes of living cells.

Examples of the use of the chemical-enzymatic method are:

- Synthesis of aspartic acid from fumaric acid (produced by *Escherichia coli*).
- Synthesis of L-phenylalanine from cinnamic acid (used by yeast cells).

The chemical-enzymatic method of producing amino acids can be illustrated by the example of obtaining L-lysine from an oil product - cyclohexene. At the beginning, D, L- $\alpha$ -amino- $\epsilon$ -capro-lactam is obtained from cyclohexene by a chemical method (Fig. 4). Then, an enzymatic reaction to obtain L-lysine from caprolactam is carried out (Fig. 6). Immobilized forms of enzymes are involved in this reaction - lactamase of *Candida laurentii* yeast and racemase of *Alcaligenes obae* bacteria.

The chemical-enzymatic method makes it possible to produce almost all amino acids, but due to disadvantages in the form of a rather expensive production process and the complexity of obtaining organic acids, the method is not economically profitable.



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#### **Biological method of obtaining lysine**

Hydrolysis of protein-containing waste is an ancient method of obtaining amino acids. During hydrolysis, white breaks down into amino acids or fragments of several amino acids, while a mixture of peptides and amino acids is formed, the isolation of which requires a lot of time.

In this method, waste from the agricultural and meat processing industry is used: horns, hair, feathers, down, which consist of keratin, which, in turn, contains a lot of the sulfur-containing amino acid cysteine.

The technology of extracting amino acids by biological method consists of preparation of raw materials, hydrolysis, purification and isolation. At the stage of hydrolysis, the prepared raw materials are subjected to the action of acids, alkalis, and enzymes. Next, the resulting mixture of peptides and amino acids is purified from fats and mineral impurities by filtration and centrifugation. The final stage of selection takes place using chromatography and/or crystallization methods.

The biological method of obtaining amino acids, including lysine, gradually lost popularity in the industry due to economic unprofitability, the difficulty of cleaning hydrolysis products, competition with more modern methods, despite the fact that the method is used locally for processing livestock waste. [12-13]

#### **Microbiological method of obtaining lysine**

The microbiological method is the most popular in the modern industrial world for obtaining lysine. The founders of this method are Japanese companies, which introduced microbiological production technologies back in the 50s of the last century. The large industrial company Eurolysine, which still produces lysine, was founded in France.

Amino acid producers in this method are industrial strains of microorganisms. In the regulation and management of metabolic processes of microorganisms, retroinhibition is used, that is, the principle of feedback, which is carried out due to the inhibition of the activity of the initial enzymes of the own synthesis of the excess product by the amino acid itself, or the complex of enzymes of the entire biochemical chain of cell metabolism is repressed, which is a natural mechanism of a living organism to maintain balance at the cellular level.

Homoserine-dependent mutants capable of synthesizing a large amount of extracellular lysine are used on an industrial scale, they belong to the genera *Brevibacterium*, *Micrococcus*, *Corynebacterium*, *Pseudomonas*, *Aerobacter*. The main species of these bacteria are *Brevibacterium flavum*, *Brevibacterium ammoniagenes*, *Micrococcus glutamicus*, *Corynebacterium glutamicum*, *Corynebacterium hoogii*, *Azotobacter suis*. Most often, auxotrophic homoserine mutants of *Corynebacterium glutamicum* and *Brevibacterium flavum* are used as a producer in the production of lysine.

Synthesis of L-lysine by microorganisms is carried out in two main ways.

Fungi, yeast and microalgae synthesize lysine

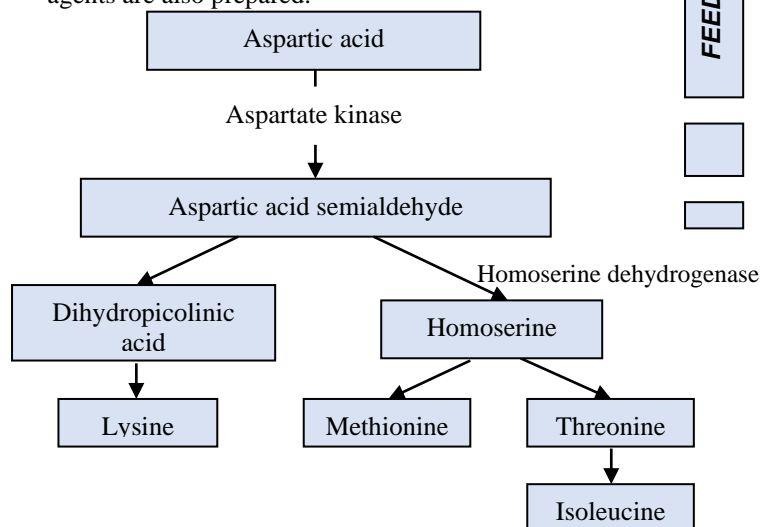
via  $\alpha$ -ketoglutaric acid using  $\alpha$ -aminoadipic acid - amino adipinate (AA) pathway. Regulation of enzyme activity at this stage is not sufficiently studied, due to which mutant forms capable of oversynthesis of lysine have not yet been obtained.

Higher plants, bacteria and some algae carry out the synthesis of lysine through  $\alpha$ -diaminopimelic acid, which begins with aspartic acid (Fig. 7) [11].

Microbiological methods of amino acid production are based on the ability of microorganisms to synthesize all L-amino acids and "oversynthesize" them under certain conditions. Industrial methods of one- and two-step synthesis are used to obtain pure amino acid preparations.

In one-step synthesis, the auxotrophic mutant is grown in an industrial incubator. [14-15]

The technological process consists of two main stages. The first stage is the preparation of auxiliary materials and nutrient media. At this stage, air and titration agents are also prepared.



**Fig. 7. Scheme of lysine biosynthesis**

The second stage is biomass cultivation and lysine biosynthesis. During the entire process, the physical and chemical parameters of the environment (pH, temperature, concentration of oxygen and nutrients), microbiological purity (absence of foreign microorganisms), concentration of biomass and concentration of lysine produced by bacteria are constantly controlled and monitored.

At the end of cultivation, the culture liquid from the fermenter is collected and processed to extract lysine. The obtained lysine is purified and dried [14, 16].

Despite the similarity of the process, different companies use specific approaches to optimize lysine production, including the main producer, different types of bioreactors and methods of purification of the final product.

#### **Conclusions**

Therefore, the cheapest and most developed way of obtaining lysine is the microbiological method.

Japan, Holland, the USA, South Korea, and China are actively implementing microbiological methods for the synthesis of lysine, which allows mass export. In Ukraine, for 2021, the total volume of lysine produc-



tion was about 50 tons/year, while the market demand was estimated at 100 000 tons. Manufacturers such as Ukrkhimprom State Enterprise, Biochem LLC, and Agrokhimprom LLC were actively working to cover domestic lysine needs until 2022, however, due to economic and infrastructural difficulties, many of these compa-

nies reduced or stopped production, which led to even greater dependence on imported lysine. To ensure food security and economic stability, the development of lysine production in Ukraine is critically needed, which will increase the competitiveness of the Ukrainian agar sector.

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## КОРМОВИЙ ЛІЗИН. ЗНАЧЕННЯ ТА СИНТЕЗ

### Анотація

Інтенсифікація тваринництва залежить від повноцінної годівлі сільськогосподарських тварин, яка передбачає вміст в кормах найважливіших поживних та біологічно активних речовин. Для одержання необхідної кількості тваринницької продукції високої якості, забезпечення високої відтворної здатності тварин, спеціалісти розробляють раціони годівлі тварин з урахуванням мінімальних витрат на корми. Забезпеченість сільськогосподарських тварин кормовим протеїном — одне з важливих завдань сільського господарства та підприємств, які виробляють білкові корми або одержують їх у вигляді побічних продуктів. Використання побічних продуктів переробки харчових виробництв має велике значення в програмах годівлі тварин, а також вирішує проблему утилізації цих побічних продуктів. Застосування таких рішень дозволить знизити потребу господарств у фуражному зерні, кормах тваринного походження та в інших дорогих компонентах, які використовують у годівлі. До білкових кормів рослинного походження відносять продукти переробки соняшнику як найдешевшого джерела кормового протеїну в Україні. Неповноцінність протеїнів соняшникової макухи та шротів слід компенсувати синтетичними амінокислотами. Лізин, як незамінна амінокислота, має широкий спектр застосування, що охоплює різні галузі від медицини до харчової промисловості та сільськогосподарської діяльності, зокрема в кормовій промисловості. Більша частина виробленого лізину використовується при виробництві кормових добавок (для свиней і птиці), які значно підвищують поживну цінність рослинних кормів з низьким вмістом лізину. Додавання лізину до складу комбікормів поліпшує засвоєність білку тваринами і знижує витрати кормів на виробництво тваринницької продукції. Враховуючи доступність лізину та беручи до уваги статистику тваринництва, а саме збільшення поголів'я свиней та зменшення поголів'я великої рогатої худоби та птиці, промислове виробництво лізину як кормової добавки для свиней є перспективним напрямком. Розглянуто основні методи виробництва лізину — хімічний, хіміко-ензиматичний, біологічний та мікробіологічний. Детально проаналізовано технологію кожного методу, наведено переваги та недоліки різних методів отримання лізину.

**Ключові слова:** тваринництво, протеїн, білкові корми, амінокислоти, лізин, методи виробництва, технологія.

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