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PHYTASE AS A FACTOR IN PHOSPHORUS ABSORPTION

Abstract

The modern world cannot be imagined without the consumption of biotechnology products. One of the key aspects of this development is the provision of high-quality and effective animal feed. Enzymes play a special role in animal nutrition, their industrial production and use takes place in many countries of the world. This is due to the fact that approximately one third of the organic matter contained in feed is not digested by the animal. Reducing these losses by 2-3% allows obtaining additional hundreds of tons of livestock products. It is especially recommended to introduce enzymes into the diet of young farm animals, whose enzymatic digestive system is not yet sufficiently developed. The biosynthesis and activity of digestive enzymes in the animal body undergo significant changes with age, growth rate, and diet composition, which results in different utilization of nutrients. The introduction of enzyme preparations into compound feeds ensures stable digestion conditions and better transformation of feed into livestock products. Today, the cost of mineral supplements is rapidly increasing. To solve the problem of optimizing energy and mineral components, increasing the availability of phosphorus and other mineral elements present in compound feeds, use the enzyme phytase. In grain feeds, a significant part of the phosphorus is chemically bound by phytates. The basis of phytates is inositol, one molecule of which connects six molecules of phosphoric acid via an ester bond. Only phytase is capable of breaking down phytates - an enzyme of plant or microbial origin, the addition of which to the diet of farm animals, including laying hens, improves phosphorus digestibility and balances the calcium-phosphorus ratio. The current state of the grain processing industry is analyzed and the feasibility of using grain processing by-products in poultry feeding is substantiated. Screening showed that phytase increases the bioavailability of nutrients contained in plant-based feeds, and also allows for a significant reduction in the level of inorganic phosphate input. The use of enzymes with different spectrums of action in feeding is an effective mechanism for regulating the mineral nutritional content of the diet by increasing the availability of protein and energy. It also makes it possible to replace expensive feed components with cheaper ones and reduce the cost of livestock products.

Keywords: enzyme preparations, bioavailability, nutrients, plant raw materials, grain processing by-products, phosphorus, phytic phosphorus, phytase, research, farm animals.

Introduction. Formulation of the problem

One of the most relevant areas of increasing animal productivity is the research and introduction of biologically active substances into the diet. Enzyme preparations play a special role in animal feeding, their industrial production and use is carried out in many countries of the world. This is due to the fact that approximately one third of the organic matter contained in feed is not absorbed by the animal. Reducing these losses by 2-3% allows obtaining additional hundreds of tons of livestock products. It is especially recommended to introduce enzyme preparations into the diet of young farm animals, whose enzymatic digestive systems are not yet sufficiently developed. [1,2]

In fact, in all cereals and legumes, the phosphorus level exceeds the calcium level by almost 5-10 times. But in poultry, according to physiological metabolism, there should be more calcium. Violation of this ratio mainly leads to impaired blood circulation, metabolism, brittle bones, liver damage, appetite distortion, and as a result - a decrease in productivity, and therefore - unprofitability of the industry.

In grain feeds, a significant portion of phosphorus is chemically bound by phytates. The main phytate is inositol, one molecule of which is linked by an ester bond to six molecules of phosphoric acid. [3] Only phytase is capable of breaking down phytates - a preparation of plant or microbial origin, the addition of which to the diet of laying hens improves the digestibility of phosphorus and balances the calcium-phosphorus ratio.

Therefore, when using wheat bran in compound feeds, it has recently been recommended to use enzyme preparations containing phytase.

The purpose of the study

The purpose of the work is to theoretically study and substantiate the feasibility of using grain processing by-products with phytase preparations to solve the problem of providing farm animals with nutrients.

Bran is a by-product of flour milling, consisting of the husk and partially the germ of the grain separated from the endosperm during flour production. This feed raw material is often a component of compound feed products.

Ukraine, as the world's leading producer and exporter of grain products, is distinguished by its diversity of agricultural crops. The grain crops grown constitute an important component of the agricultural sector.

According to data from the Ministry of Agrarian Policy and Food of Ukraine for 2023, the main grain crops in terms of cultivation are corn, wheat, barley, and oilseeds - sunflower, soybean, rapeseed (Fig. 1). [4]

The development of the food industry is a key element in ensuring the country's food security. Ukraine has a well-established food culture, where flour milling industry products play an important role.

The dynamics of flour production in Ukraine has been declining in recent years, which is associated with a decrease in exports, low profitability of production, a decrease in demand, changing gastronomic tastes of Ukrainians, and a decrease in the population. By 2022,

the production of wheat and wheat-rye flour had decreased by 37%, and exports by 76%. [5]

The total volume of wheat exported to the world market in the 2022/2023 MY amounted to 212.9 million tons, which is the highest in history. Ukraine's share in world exports is 7%, which ranks sixth in the world (Fig. 2). [4]

Fig. 3 shows some importing countries of Ukrainian wheat. [5]

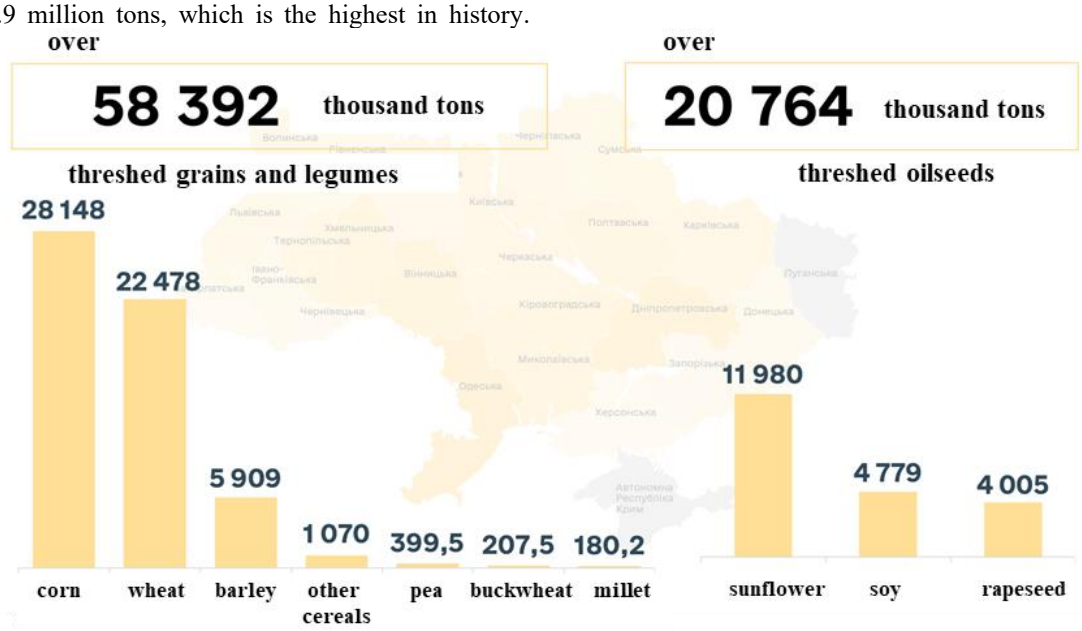

Fig. 1. Volume of grain and legume cultivation in Ukraine

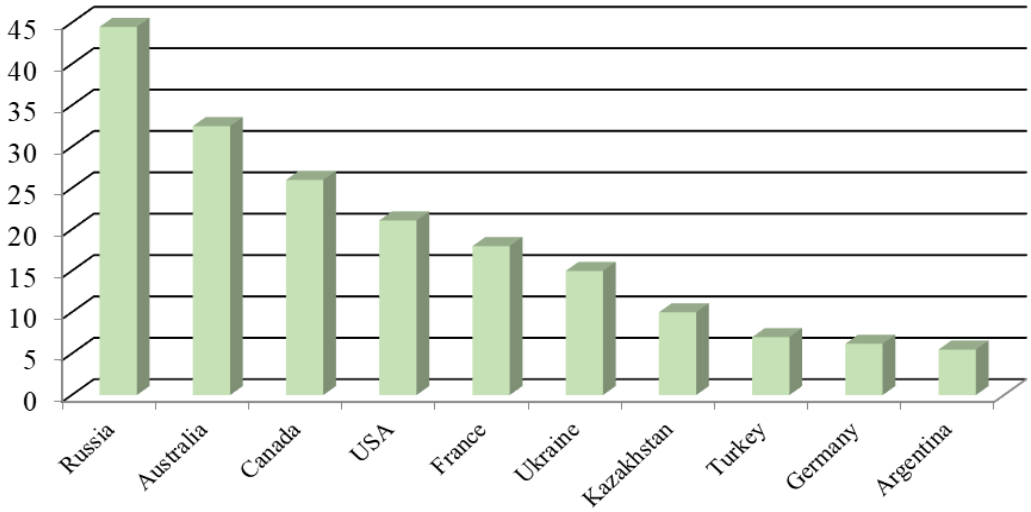

Fig. 2. TOP 10 wheat exporting countries in the world 2022/23 MY

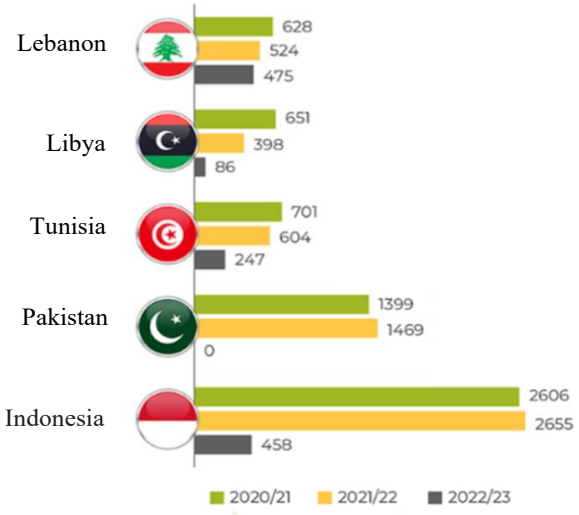

Fig. 3. Volume of exports from Ukraine by countries importing Ukrainian wheat, thousand tons

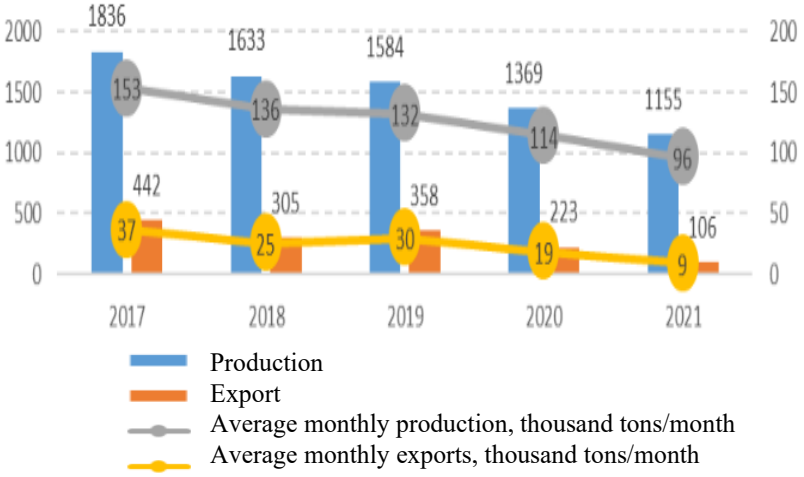

Fig. 4. Dynamics of production and export of wheat and wheat-rye flour in Ukraine, thousand tons

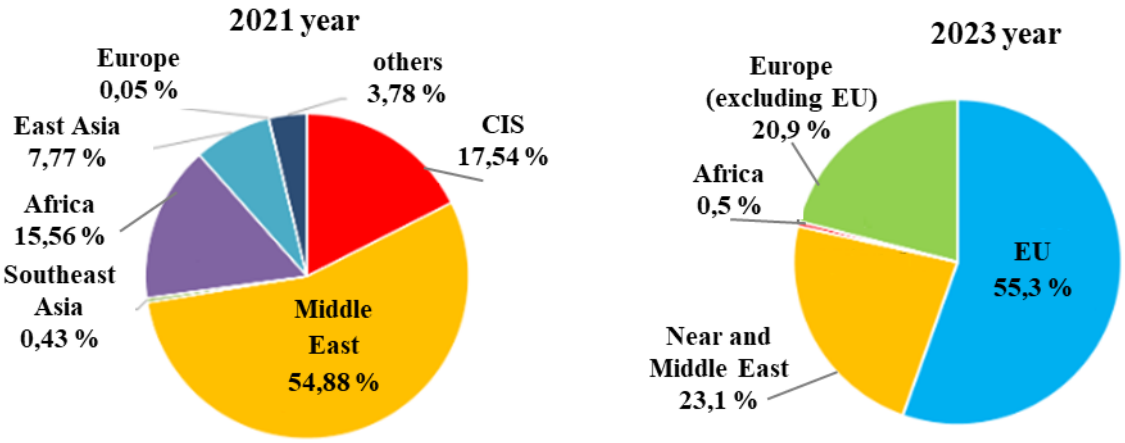

Fig. 5. Geography of Ukrainian flour exports, %

The geography of Ukrainian flour exports has changed significantly: exports to the EU exceeded 50% of the total volume sold (Fig. 5). [5]

A certain restraining factor for increasing flour production is also the sale of bran. Taking into account the additional assessment of flour production and the estimated yield of bran in flour milling production, by 2022 an average of 85% of the formed bran stocks were exported. At the same time, despite the constant decrease in flour production, the dynamics of bran exports remained relatively stable (Fig. 6). In most cases, it was the export of bran that provided positive profitability for flour milling enterprises. [5]

An important stage in flour milling production is ensuring the sale of bran, since the formation of significant stocks at enterprises technologically does not allow the production of flour. The main importer of Ukrainian bran is Turkey, which purchases an average of 90% of all exports. Therefore, due to the lack of shipments at ports due to the Russian military invasion, these volumes will remain at the enterprises.

Wheat bran is rich in phosphorus, but its absorption by animals is very low, since phosphorus in bran (Fig. 7), as in most plant types of feed raw materials, is contained in the form of phytate compounds. Such compounds are difficult to hydrolyze in the gastrointestinal tract of the animal.

Only phytase is capable of breaking down phytates - an enzyme preparation of plant and microbial origin, the addition of which, for example, to the diets of laying hens, improves the digestibility of phosphorus and balances the calcium-phosphorus ratio. Only ruminants can effectively absorb wheat bran phosphorus without exogenous enzymes, since phytate phosphorus is easily hydrolyzed by phytase, which is produced by rumen microflora.

Phosphorus is a significant part of the lithosphere. Heterotrophic organisms obtain phosphorus from plants, in which this element is found mainly in the form of phytates. Phytates are calcium and magnesium salts of inositol hexaphosphoric acid (Fig. 8).

Phytates account for up to two-thirds of all phosphorus in the human diet, with one-third represented by inorganic phosphorus, which is easily absorbed.

However, phosphorus in phytates is unavailable to humans and many farm animals (cattle, pigs, poultry) due to the absence or insufficient amount of digestive enzymes that cause phytate degradation. In addition, phytates, which are negatively charged, bind calcium, zinc and iron ions, which significantly reduces the absorption of these important mineral elements in the intestine (Fig. 9). This feature of phytates is one of the causes of iron deficiency, which affects more than 3 billion people on the planet. The formation of protein complexes with phytates impairs the nutritional value of feeds.

Typically, in conditions of low activity or complete absence of phytases, phytic phosphorus and the beneficial nutrients associated with it pass through the gastrointestinal tract in transit. This reduces the availability of phosphorus in grain feeds to 15-22% of its content

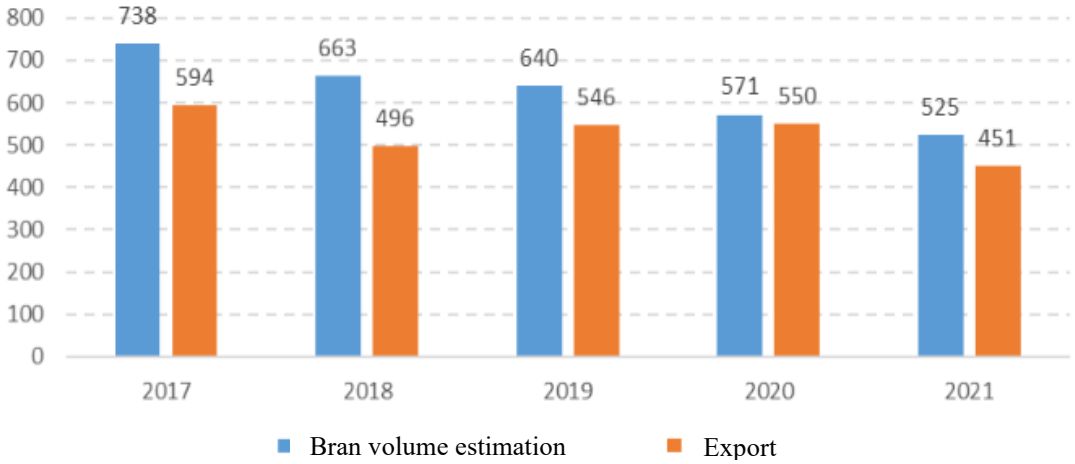

Fig. 6. Volume of bran formation and its export in Ukraine, thousand tons

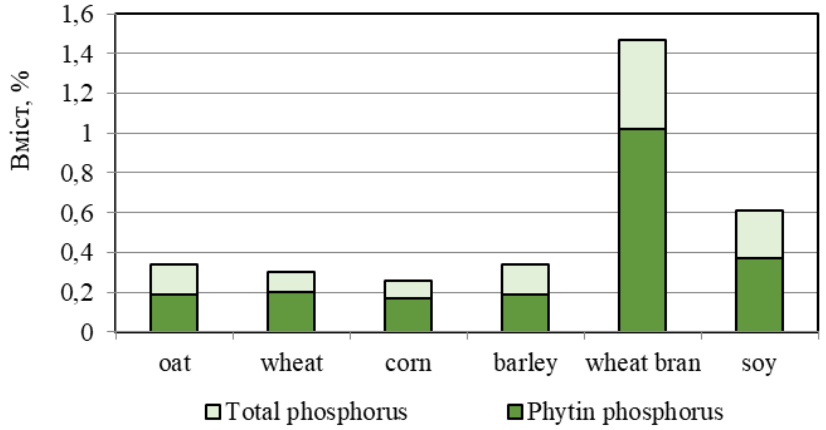

Fig. 7. Content of total and phytic phosphorus in various plant feeds

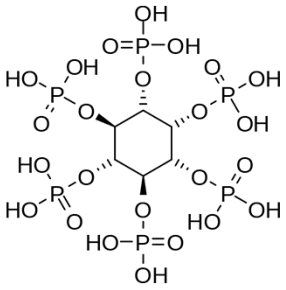

Fig. 8. Inositol hexaphosphoric acid

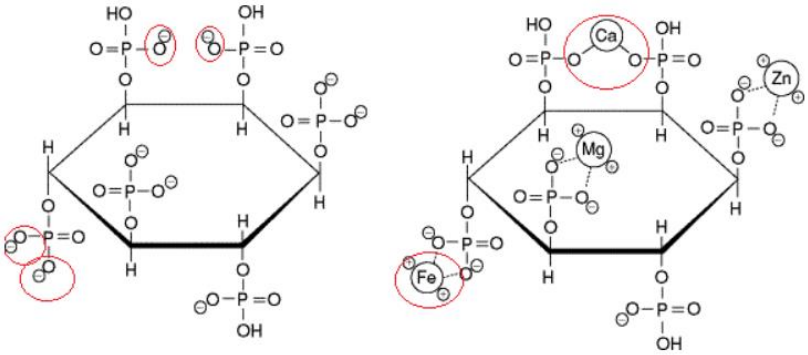

Fig. 9. Structural formulas of phytates

in the feed, and the degree of utilization of associated minerals is reduced by 8.7-25.8%. [6,7] Phytic phosphorus, passing unchanged through the digestive tract of animals, is excreted in the manure, which is applied to the soil as an organic fertilizer.

A huge amount of unabsorbed phosphorus is excreted by animals into the environment. This fact represents a significant environmental problem, as it leads to eutrophication of water bodies and, as a consequence, a detrimental effect on living organisms.

That is why interest in enzymes that catalyze the cleavage of inorganic phosphorus from phytates is growing.

In a biological system, the hydrolysis of phytic acids to myoinositol and phosphates is considered an important reaction in energy metabolism, signaling, and regulatory pathways of metabolism.Phytic acids and their degradation products are involved in the regulation of cell proliferation and differentiation, control of blood glucose and cholesterol levels, antitumor processes, and treatment of Parkinson's and Alzheimer's patients.

Phytases belong to the class of hydrolases and subclass of phosphatases that catalyze the hydrolysis of monoesters of phosphoric acid. Phytases (myo-inositol-1,2,3,4,5,6-hexakisphosphate phosphohydrolases) release at least one phosphate ion from the phytic acid molecule. Hydrolysis of phytic acid produces lower inositol phosphates, which contain fewer than six phosphoric acid residues, inositol, and inorganic phosphate, and releases phytate-bound cations. [8]

According to the International Nomenclature of Enzymes IUPAC-IUBMB, three types of phytases are distinguished: 3-phytases (KE 3.1.3.8), 5-phytases (KE 3.1.3.72) and 4/6-phytases (KE 3.1.3.26).

Phytases of different types begin the transformation of phytic acid by hydrolysis of the ester bond at different carbon atoms of the inositol ring, resulting in the formation of different isomers of lower inositol phosphates (Fig. 10). The mechanism of phytate hydrolysis and the structure of protein molecules of phytases of the same type may differ.

3-phytase is characteristic of microorganisms, 4/6-phytase of plants. [9]

5-phytase was relatively recently discovered in plants belonging to the legume family (alfalfa and beans) and in the bacteria of the genus Selenomonas ruminantium. However, there are some exceptions: soybean phytase is a 3-phytase, and E. coli phytase is a 6-phytase. [10, 11]

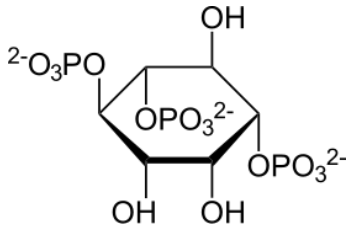

Fig. 10. Inositol triphosphate [12-14]

When choosing a preparation containing phytase for feeding farm animals, one should pay attention to the following properties of the enzyme:

- Enzyme activity;
- Specificity of action and mechanism of hydrolysis of phytic acid: 3-, 5- and 6-phytases;
- Animal, plant or microbial origin;
- Producer microorganisms;
- Optimum temperature and optimum pH of the enzyme;
- Effect of heat treatment in the production of compound feed products;
- Resistance to the action of proteases of the animal's gastrointestinal tract. [15]

Based on the pH optimum, phytases are divided into acidic and alkaline.

Phytases are actively produced by soil microorganisms and participate not only in the destruction of fresh organic residues, but also in the release of phosphorus from soil organic compounds. Thus, microbial phytases are key enzymes in the cyclic turnover of organic phosphorus in the soil.

Microbial phytases can be classified as acid, neutral, or alkaline phosphatases, depending on their optimal pH level.

Today, enzymes with phytase activity are divided into:

- histidine acid phytases (HAPs);
- 3-propeller phytases (BPPs);
- purple acid phytases (PAPs);
- cysteine or protein-tyrosine phytases (PTPs).

Representatives of each of these enzyme groups have different catalytic mechanisms and individual properties that allow them to effectively utilize myo-inositol hexaphosphates as a substrate at different pH values.

Among microorganisms, the most active producers of extracellular phytases are micromycetes of the genera *Aspergillus, Penicillium, Misor*, and *Rhizopus.*

In bacteria, phytases were found in *Aerobacter aerogenes, Bacillus subtilis, Klebsiella sp., Pseudomonas sp., Enterobacter sp. 4, Escherichia coli, yeast Saccharomyces cerevisiae and Candida tropicalis, Torulopsis Candida, Debaryomyces castellii, Debaryomyces occidentalis (a synonym of Schwanniomyces castellii), Kluyveromyces fragilis.*

In general, bacterial phytases produced by representatives of the genera *Bacillus* and *Enterobacter* have a pH optimum in the range from 6.0 to 8.0, and a temperature optimum ranging from 45 to 77°C. A. niger phytase with a pH optimum of 5.0-5.5 is not thermostable and is not capable of refolding after thermal denaturation, unlike another phytase from this producer with a pH optimum of 2.2, which loses some activity at 80°C.

High thermal stability of fungal phytases was observed in the recombinant enzyme *A. fumigatus*. Phytases of *A. niger* and *E. coli*, in comparison with recombinant phytases isolated from wheat, are more resistant to proteolytic degradation by pepsin than others.

Many microbial phytases have already been studied and are used in various agro-industrial fields. The effectiveness of these enzymes in feeding and in the process of digestion in the animal body depends on their ability to maintain their potency regardless of the different conditions that may occur at different stages of feed preparation and in the digestive tract. The preparation of these feeds requires heat-resistant enzymes, while the pH range is, for example, from 5.02, 2.75, 6.28, 6.63 to 5.98 during passage through the goiter, stomach, duodenum, jejunum, and ileum. [15]

The global phytase market is estimated at around $350 million per year. On average, around 70% of all pig feeds and around 90% of all poultry feeds contain phytase.

The growth of the phytase market is driven by two main factors: the need to replace inorganic phosphates in animal diets due to their increasing cost and the impact of livestock farming on the environment, especially the need to minimize phosphorus emissions [16].

Phytase Novo is an enzyme that breaks down phytic acid complexes and is used to improve the absorption of phosphorus from all feeds. This reduces the phosphorus content in feeds by approximately 30%. This enzyme improves the absorption of trace elements from plant raw materials associated with phytic complexes, increases animal immunity and reduces feed consumption.

The rate of introduction of the drug Phytaza Novo into premixes for broilers and pigs is 20 kg per 1 ton of premix, and for laying hens - 12 kg per 1 ton of premix.

Distinctive features of Novo Nordisk enzyme preparations are high stability during storage (microgranulator – up to 12 months), storage as part of vitamin and mineral premixes (up to 12 months without loss of activity), stability during granulation (compound feed is heated to a temperature below 82°C, conditioning time is less than 25s) [17-20].

BASF produces phytase preparations under the trademarks Natuphos™ 5000 (powder), Natuphos™ 5000 L (liquid), Natuphos™ 5000 G and Natuphos™ 5000 G (EU) (granulated powder), which are characterized by a phytase activity of at least 5000 FTU/g, a wide range of pH values of 2-6.5.

Natuphos™ 10000 G is particularly suitable for use in concentrated premixes. Shelf life 12-15 months, for NatuphosTM 5000 L – 6 months. Dosage rates: piglets, fattening pigs, breeding sows, broilers, turkeys – 500 FTU/kg, laying hens – 300 FTU/kg [17].

BASF produces complex enzyme preparations on an industrial scale under the brands Natugrain™ Blend G and Natugrain™ Blend L. This product is a highly effective and versatile poultry product used in wheat-based feeds with up to 30% barley. This complex with β-gluconase and β-xylanase activity is also effective in diets containing wheat bran and meal. Shelf life up to 6 months. Recommended doses are 100 g/t and 150 g/t respectively.

Adisseo (France) produces an enzyme preparation for poultry called ***Rovabio™ Xylan***, which has xylanase activity. The rate of introduction of the preparation into the premix composition is 5 kg per 1 ton of premix. The level of energy metabolism of the rations increases by 12-15% [17].

Hoffman La Roche (Switzerland) produces the multienzyme preparation ***Roxazyme G*** to increase the efficiency of digestion of nutrients in grain components of compound feeds for poultry and pigs (the rate of introduction is 8-15 kg per 1 ton of premix), the enzyme preparations ***Ronozyme WX CT*** and ***Ronozyme A CT*** to improve the digestibility of wheat, wheat-barley diets for poultry and pigs (introduction rate – 15-25 kg and 5-10 kg per 1 ton of premix), ***Ronozyme VP CT*** for diets with a high content of sunflower and soybean meal (introduction rate – 25-40 kg per 1 ton of premix), ***Ronozyme P CT*** to improve the absorption of phosphorus, calcium and microelements from plant feeds (introduction rate – 18-30 kg per 1 ton of premix) [17].

The enzyme preparation ***Ladozym "Proxy" F*** is used as a feed additive in the feeding of farm animals and poultry; this preparation contains a complex of enzymes, the main one of which is phytase [21].

The use of the enzyme preparation Ladozym