Research Article

iMedPub Journals
www.imedpub.com

Journal of Plant Sciences and Agricultural Research

2021
Vol. 5 No. 4: 56

The Results of Studies of the Activity of Feed Enzymes *In Vitro* Depending on the Temperature and pH of the Medium under Conditions Simulating the Gastrointestinal Canal of Poultry

VS Kozyr[1*], OP Reshetnychenko[2], PP Antonenko[3], RV Mylostyvyi[3], IV Glebova[4], VS Kryukov[5], SV Zinoviev[6]

1 Institute of Grain Crops NAAS, Dnepr, Ukraine
2 Odessa State Agrarian University, Odessa, Ukraine
3 Dnieper State Agrarian and Economic University, Dnieper, Ukraine
4 FSBEI HE Kursk State Agricultural Academy, Kursk, Russian Federation
5 LLC Vetfarmstandar, Moscow, Russian Federation
6 FRC All-Russian Scientific Research and Technological Institute of Poultry RAS, Sergiev Posad, Russian Federation

*Corresponding author: VS Kozyr

izkzoo3337@gmail.com

Institute of Grain Crops NAAS, V. Vernadsky St., Dnepr, Ukraine.

Citation: Kozyr VS (2021) The Results of Studies of the Activity of Feed Enzymes *In Vitro* Depending on the Temperature and pH of the Medium under Conditions Simulating the Gastrointestinal Canal of Poultry. J Plant Sci Agri Res Vol.5 No.4:56

Abstract

Aim: To study the activity of enzymes of the gastrointestinal tract of different manufacturers at different temperatures and acidity in the conditions of an in vitro model fermenter.

Methods: In a certified laboratory to determine the activity of enzymes using biochemical, physiological, analytical, metrological methods.

Results: The activity of endo-1.4-glucanases and endo-1.4-xylanases was studied in 24 feed enzyme preparations in the conditions in vitro simulating the gastrointestinal tract of poultry. Lowering the temperature of the medium from 50°C to 38°C negatively affects the activity of all enzymes, which at pH 3.0 was almost 2 times higher than at pH 7.0. In general, the activity of enzymes containing xylanase was more stable compared to glucanase under conditions simulating both the stomach and intestine.

Conclusion: The results obtained in the experiments in vitro allow us to conclude that the matrix values of enzyme activity indicated by manufacturers when labeling products do not reflect its effect in the organism of animals. Therefore, feed enzymes presented on the domestic market should be used only after scientifically based production studies. When developing recipes for compound feeds and premixes, which include enzyme preparations, it is also necessary to take into account the specific effect of feed enzymes on the type of raw material, changes in the digestive system in ontogenesis, and the functional features of various sections of the gastrointestinal tract of animals.

Keywords: Premix; Feed enzymes; Activity; Matrix values of enzymes

Received: March 19, 2021; **Accepted:** April 02, 2021; **Published:** April 09, 2021

Introduction

Among synthetic feed enzymes, phytase was created specifically for use in animal feed. All other enzymes were originally developed for industrial use, and were subsequently proposed as feed additives. However, manufacturers almost do not take into account the peculiarities of animal's digestion. Therefore, the drugs they offer are not always effective and exhibit maximum effect under conditions far from corresponding to the medium into which they enter in the Gastrointestinal Tract (GIT). The predominant purpose of enzymes for industrial purposes is confirmed by methods for monitoring their activity, which in the vast majority of cases is determined at a temperature of about 50°C-55°C and pH of 5.0-5.5. These conditions correspond to industrial ones, where the temperature is much lower than technological regulations (not 50°C-55°C, but 42°C) and the environment is different (pH is not 5.0-5.5 but 3.0). In addition, in poultry, they change along the goiter-stomach-intestine. Moreover, in the digestive tract there are own digestive enzymes that digest proteins and can digest added enzymes that have different resistance to proteases. Therefore, we believe that our research is relevant [1].

The aim of the investigation was to study the activity of enzymes of the gastrointestinal tract from different manufacturers at

different temperatures and acidities in the conditions of the model fermenter *in vitro*.

Materials and Methods

The research material was 24 of the most common Enzyme Preparations (EP) produced in different countries. Under the conditions of modeling the medium of the gastrointestinal tract of poultry (pH 3.0+pepsin or pH 7.0+trypsin, 1.38°C), the activity and stability of endo-31, 4-glucanases (glucanases) and endo-31,4-xylanases (xylanases) of various commercial feed EP, which are widespread in Russia and intended for the digestion of NSP.

The effect of Gastric Juice (GJ) on the stability of enzymes was studied in an incubation medium containing 41.7 units/ml of pepsin. A mixture of the studied EP preliminarily diluted to a certain level with GJ was incubated at 38 ° C, samples were taken from the reaction mixture (30 min and 120 min) and the activity of endoglucanase and xylanase was determined in relation to the water-soluble stained with the orange derivative of Carboxymethyl Cellulose (CMC) and birch xylan. In addition, the activity of the ferments of the preparations with respect to these substrates was determined at pH 5.0 and temperature 50°C [2].

Of the ferments of the Intestinal Contents (IC), the most active on feed proteins, including the proteins of feed enzymes, are proteases, and primarily trypsin. Therefore, it was chosen to create an IC model and test its effect on enzyme stability. The effect of IC on the stability of EP enzymes was studied in an incubation medium containing 3.125 units/ml of trypsin.

A mixture of a solution of the test drug with intestinal contents previously diluted to a certain level was incubated at 38°C. Samples were taken from the reaction mixture (30 minutes and 120 minutes) and the activity of endoglucanase and xylanase was determined in them using CMC or birch xylan soluble in the aqueous medium as substrates. The activity of EP enzymes with respect to these substrates was also evaluated at pH 5.0 and temperature 50°C.

Results and Discussion

When studying 24 enzymatic preparations, it was found that the activity of endo-β-1,4-glucanases and endo-β-1,4-xylanases are 57.3% and 42.8% lower, respectively, against activity at pH 5 and a temperature of 50°C. In some preparations, the activity of glucanase in the conditions of stomach was 75%-

Table 1. The activity of enzymes from different manufacturers at pH 5 and a temperature of 50°C, compared with pH 3 and a temperature of 38°C [1-6] under conditions simulating the gastrointestinal canal of poultry.

Feed enzymes	Country of origin	Activity at 50°C pH 5, u/g		Activity at 38°C in % is determined at 50°C, pH 5			
		Glucanase	Xylanase	Glucanase		Xylanase	
				pH 3.0	pH 7.0	pH 3.0	pH 7.0
Fekord-2004-S	Belarus	80	290	90	33	81	47
Agrocell Plus	Russia	4100	1050	56	32	80	42
Acra XB 201 TPT	USA	1300	780	62	11	62	45
Econase HT 25	Germany	65	2100	63	13	54	47
Agroxil Plus	Russia	1100	4100	60	35	54	32
Agroxil Premium	Russia	3200	2700	56	36	54	42
Vilzim	Mexico	2180	11000	58	12	52	28
Agrocell	Russia	4200	1040	40	25	70	45
Rovabio Max AR	France	1480	2720	23	4	70	8
Xubeten-Kel	Bulgaria	4000	610	25	5	67	0
Extra XAP 101 TRT	Finland	-	1800	-	-	67	52
Endophyte DC	Spain	580	1000	31	6	65	26
Cellulases	China	5870	500	39	31	62	5
Cellolux F	Russia	3500	1440	23	31	61	7
Agroxil	Russia	1100	5100	44	38	60	25
Roxazim G2G	Switzerland	350	720	46	32	58	17
Rovabio Exel AP	France	1490	2850	20	6	57	4
Xubeten -Xil	Bulgaria	1600	4000	33	32	56	10
Fekord -2012-F	Belarus	400	200	52	27	47	32
Sunzaime	China	1450	4100	34	28	46	8
Hostazim S-100	Bulgaria	500	100	40	52	44	17
Ronozyme VP	Denmark	130	150	35	37	42	29
Natugrain TS	Germany	225	1980	43	-	40	-
Ronozyme WX	Denmark	15	970	-	30	38	-
On average for all enzymes		-	-	44.2	19.1	57.8	30.6

80% lower than at pH 5 and a temperature of 50°C, and in 6 of them it decreased by 35%-45%. And only in one drug by 10% (Table. 1).

The activity of endo-β-1,4-xylanases of the same drugs in the stomach conditions was higher than glucanase. Moreover, its decrease in xylanase was not symbiotic with respect to glucanase: in some cases, it decreased more progressively, and in others, on the contrary. When kept in an acidic medium in the presence of pepsin for 30 minutes and 120 minutes, the activity of the enzymes continued to decrease, but in most drugs to a lesser extent than initially under the influence of a lower temperature. Perhaps this was due to the digestion of enzymes under the influence of pepsin or caused by the denaturation of their protein in an acidic medium. The activity of the studied enzymes at pH 7, which is close to the intestinal environment, where most of the time the fodder masses are located and are digested, turned out to be significantly lower than in the acidic medium of the stomach [3].

In recent years, scientists and specialists in different ways reflect the concept of enzyme activity and their action. At the same time, attention is drawn to the fact that the units characterizing activity are provided for the purpose of marking commercial preparations and have no value for comparing their properties. This creates certain confusion in the reflection of activity by units having different dimensions. So, only to indicate xylanase activity, 9 different units are known. The enzyme activity indicated by the manufacturer characterizes its ability to perform a certain action in specific standard conditions of analysis, which always differ from the conditions prevailing in the body. Therefore, the activity established by the manufacturer does not coincide with its manifestation in the DT and is not a reliable way to identify the comparative effectiveness of feed enzymes [4].

Activity is characterized by the amount of product resulting from the action of enzymes on the substrate. It depends on the measurement conditions, including the pH of the medium, its composition, temperature, substrate used, incubation time, medium mixing intensity and other parameters. In standardized *in vitro* measurement conditions, a clean, free substrate is used, while in feed they are not present in pure form, since they are embedded into the structure of cells, which makes their availability for enzyme action more difficult. Throughout the DT, the pH and composition of the medium, the concentration of available substrate, change. As a result of evolution, animals have such conditions for the digestion of food that, as it moves along the DT, new enzymes are secreted, and not one enzyme acts throughout its entire length. This is especially evident in the case of proteases. Pepsin acts on proteins in the stomach and is not active in the intestine, in which protein digestion continues under the influence of trypsin and then peptidases. The feed enzymes used have specific properties, are active in certain conditions and are not adapted for action on all parts of the digestive system. In this regard, the activity of commercial enzyme preparations, measured in an *in vitro* model solution, cannot be used to rank by the effectiveness of their action in the DT. According to the instructions for the use of feed enzymes, their activity is indicated on the basis of the declaration of the manufacturer (supplier) in the form of units, without indicating their dimension. Conclusions about the effectiveness of drugs can only be made on the basis of animal test results, which can be reproduced in future if the enzyme is used in similar conditions. The main ones are the composition of the diet and the age of the animals. Failure to comply with these requirements leads to conflicting conclusions about the effectiveness of the same enzyme. It is unacceptable to transfer the results to enzymes of the same purpose, but purchased from different manufacturers, since they can be different [5].

Many scientific publications report that the use of feed enzymes stimulates feed intake. This conclusion is in most cases true if they are used in the background of reduced energy or protein. With a sufficient level of energy, an increase in its availability as a result of the action of enzymes, will lead to a decrease in feed intake and, possibly, a lack of other substances [6].

The effectiveness of enzymes depends not only on the ability to digest target substrates, but also on the amount of other nutrients that, when bound to substrates, cannot be digested. There are a direct mechanism and indirect ones by which enzymes that digest Non-Starch Polysaccharides (NSP) improve productivity. The first weakly affects the additional supply of energy to the body. The most important is the indirect effect, which is associated with a decrease in the anti-nutritional properties of non-starch polysaccharides as a result of their decomposition. This eliminates the encapsulating effect of the cell wall and reduces the viscosity of the chyme. As a result, the accessibility of pancreatic enzymes to intracellular starch, which is the main source of energy, increases. The breakdown products of cell wall polysaccharides, partially represented by monosaccharides, are absorbed while oligosaccharides possess probiotic properties. They are converted by microflora to volatile fatty acids, which are absorbed and are a source of energy. In addition, they lower pH that inhibits the growth of coliform bacteria. At the same time, butyric acid is formed, which promotes the growth of microvilli of the brush border [6]. Moreover, in general, the population of microorganisms is reduced, which leads to a decrease in their intake of nutrients, increasing their availability for the animal.

Given the diversity of the structure and composition of plant feed cells and the properties of enzyme preparations, it can be assumed that some combination of enzymatic activities will be effective. It is rather difficult to choose enzymes for combination in a multi-enzyme preparation. Any enzyme preparation with the intended purpose, in addition to increasing the availability of its substrates, partially overcomes its anti-nutritional effect [7]. The response to enzyme use is always multifactorial. If we exclude the extreme case of inactivation of enzymes, then they always contribute to the digestion of those substances that they are aimed at, that is, they show their properties. The lack of zoo-technical results can be associated with an unsuccessful choice of enzymes, a violation of the technology of their use or the

imbalance caused by them the nutrients entering into the body.

Animals digest from 75% to 80% of the organic matter of the feed. The remaining inaccessible part is the target of impact of enzyme preparations, i.e. feed enzymes or poly-enzyme preparations are designed so that they are aimed at the indigestible part of the feed. The composition of this fraction varies depending on the components of the diet and the physiological peculiarities of digestion. Therefore, for a more informed choice of feed enzymes, it is necessary to have a characteristic of the undigested part of the feed.

When choosing enzymes based on their specificity, they often lose sight of the features of their action in the DT, which are accompanied by additional effects not related to their specificity. Enzymes have to be selected based on the current availability of feed raw materials, as well as the expected content of substrates and age-related digestion peculiarities.

The market of feed enzyme preparations is striking in variety, which creates difficulties in terms of elucidating their action and interaction in the DT, in order to predict productivity. For example, when poly-enzyme preparations were introduced, their effect was no better than for products containing one of the tested enzymes. Although there are reverse examples [6]. Such a regularity is known that confirms diminishing returns when each new activity is added into a multi-enzyme preparation. Therefore, it is impossible to predict the effect of multi-enzyme additives by summation the effectiveness of each enzyme.

In practical conditions, the effectiveness of specific enzyme preparations is characterized by the magnitudes of increase in the availability of exchange energy, amino acids, phosphorus, calcium from feed, that is, matrices. Matrix values vary depending on the type, activity of the enzyme, age and species of animals, as well as the composition of the feed. In order to choose and apply the enzyme preparation correctly, one has to understand how the values presented in the matrices are substantiated and how real they are. The development of matrices is a difficult task, because, as described above, enzymes are characterized not only by their direct effect on target substrates, but also by indirect influence. A significant range of variability of the values characterizing the cleavage of substrates even for the same type of grain significantly complicates the forecast for an increase in the availability of nutrients. From this it follows that it is impossible to reflect the effect of the enzyme on different types of raw materials with a single matrix for a particular preparation, the shares of which in mixed feeds are variable. Carrying out experiments on pigs, scientists came to the conclusion that the use of a matrix with a fixed phosphorus value for different types of diet is not recommended, as this can lead to the development of an inadequate diet [8].

In scientific studies, in some cases, there was a lack of advantages of the tested enzyme compositions compared to drugs with a single activity. Thus, the inclusion of 2-3 enzyme preparations into the compound of feed recipe on the basis of information about the action of each one individually in order to increase productivity is not always substantiated.

Effective combinations of activities must be established in scientific experiments and then confirmed in practical terms. Based on the work performed, matrix values of the action of poly-enzyme preparations can be determined, which will be inherent only to a specific combination of activities and only for feeds with a composition close to that used in the development of a recipe for a poly-enzyme preparation.

When developing recipes for compound feeds using computer programs, matrix values of enzyme preparations are often used, which are entered into the raw material database. There are two ways to represent matrix values that reflect the action of enzymes: the first is by influence, and the second is by composition. The first method reflects the magnitude of the increased availability of feed nutrients and is most often expressed as a percentage. In this case, it is advisable to calculate the effect of the enzyme separately on the main types of raw materials (wheat, corn, meal and other components). The second way is by composition, easier for the developer of the recipe, because it involves changing of the nutritional value of the entire diet. In the second method, the matrix values are represented by virtual values, suggesting that from 0.1 g-0.5 g of the enzyme per kilogram of feed, 60 kcal/kg-100 kcal/kg Eex, 40 mg-60 mg of available lysine, 20 mg-50 mg of available methionine, 600 mg-1100 mg of phosphorus will be added. Naturally, these amounts are not added to the feed with a small dose of the enzyme. It is assumed that the availability of food nutrients will increase by such values when the enzyme is included into it. In our opinion, this method, with its simplicity, has a serious drawback: it is worse adapted for the accounting changes in the ratio of the main raw materials in the recipe, and therefore there is a higher risk of obtaining an inadequate recipe. Application of this method creates involuntary or intentional "mistakes" of premix sellers. So, when an enzyme is included into a premix containing synthetic amino acids that are free, that is, have 100% availability, and the developers of a premix recipe often impose the influence of the enzyme matrix on it. As a result, the content of amino acids in the premix is expressed in higher values compared to the actual amount included into its composition. The use of enzyme matrices in the calculation of premix recipes is erroneous, since they lack substrates that enzymes can affect. If we return to the definition of the concept of a matrix, then this word, put into circulation by businessmen to promote enzymes, did not add anything new to their properties, and it is quite possible to do without it. The nutritional tables of raw materials are also its matrices. We can say: the nutritional matrix of wheat, oilcake, etc., despite the fact that their nutritional value is repeatedly determined. Each time, when a new batch of raw materials arrives, feeding specialists send it to the laboratory to establish the actual composition, that is, to elaborate the nutrition matrix. This technique has become an erroneous unwritten rule. Why constantly use enzyme matrices that were once determined somewhere or simply calculated and not subject to elaboration? And how needful are such matrices? The official instructions on

the use of enzymes provide data on the composition of enzyme preparations and their activity, as well as recommended doses of the drug. But, in no instruction do matrixes of enzymes are given, because developers, unlike sellers, cannot indicate their fixed values. After all, the values of the matrices are unofficial and are suitable only for advertising purposes. Materials and test reports used in matrix design should be available to consumers. The results of their development should be presented to customers and based on many replicates. In this case, results will be obtained in a certain range of oscillations. This is natural, and the developer should explain to the client why in some cases the effect of the enzyme preparation was high, and in others it was weak, and how to achieve the greatest efficiency. The maximum results obtained by the developer under ideal conditions are a target for the consumer that can be achieved, but it is not guaranteed. To achieve the greatest result, it is necessary to turn not to the matrices, but to the recommendations of scientists.

In recent years, livestock farmers include 2-3 enzyme preparations into the diet. At the same time, there are misunderstandings regarding the assessment of their possible effectiveness and errors in predicting the expected productivity. The effect of the addition of each new enzyme depends on the amount of substrate present in the feed. In addition, one enzyme, for example, cellulase, destroying the cellulose of cell membrane, increases the possibility for the action of other enzymes, the substrates of which are inside the cell. However, the xylanase and pectinase destroy the cell membrane; therefore, any of the enzymes that destroy the cell membrane in other ways will reduce the possibility of the manifestation of this effect by another enzyme. None of the calculations can predict the effect of multi-enzyme drugs or several drugs in the diet.

The most widespread use of enzymes was pointed in growing of young animals. However, it is difficult to predict the effectiveness of their action, which is due to active changes in the digestive system at an early age. It is especially difficult to develop the “right” matrix characterizing any enzyme for broiler chickens and piglets after weaning. On the example of changes in the use of energy of exchange energy from the same wheat, it was found that in 7-day-old chickens the Eex value was 2637 kcal/kg. At 21 days of age it was 2748 kcal/kg, and at 35 days 2933 kcal/kg. A significant increase in Eex with age shows that the digestive system at this time is actively changing, achieving optimal extraction of nutrients from feed by 35 days. When developing diets, they usually use the only Eex value taken from reference books or calculated on the basis of nutrient digestibility coefficients established in balance experiments at any one age. This approach to the calculating the nutritional value of the feed and the influence of enzymes on it does not guarantee success in predicting productivity [9].

Supplier promises without verification of the results by test reports should not be taken into account. Subject to the above requirements, enzymes always show a positive effect, however, the real value of the effectiveness of their use can be established only as a result of preliminary tests of the drug in specific conditions [10].

Conclusion

The enzyme activity value indicated by the manufacturer is intended only for product labeling and does not reflect its actual effect in the animal body. Use feed enzymes only after evidence-based production research. When developing recipes for compound feeds and premixes, take into account their specific effect on the type of raw material, changes in the digestive system during ontogenesis, and functional features of various sections of the gastrointestinal tract of animals.

References

1. Volchok AA, Korotkova OG, Kondratyeva EG, Kryukov VS, Sinitsyna OA et al. (2018) The activities of glucanases and xylanases from enzymatic feed additives in the gastrointestinal tract in poultry. Ptitsevodstvo 4: 39-45.
2. Almeida, F. N Vazquez-Añón, M, Escobar J (2017) Dose-dependent effects of a microbial phytase on phosphorus digestibility of common feedstuffs in pigs. Anim Biosci 30: 985.
3. Bedford MR (1996) The Effect of Enzymes on Digestion1. J Appl Poult Res. 5: 370-378.
4. Bedford MR, Schulze H. (1998) Exogenous enzymes for pigs and poultry. Nutr Res Rev. 11: 91-114.
5. Ingelmann C-J, Witzig M, Möhring J, Schollenberger M, Kühn I, et al (2018) Effect of supplemental phytase and xylanase in wheat-based diets on prececal phosphorus digestibility and phytate degradation in young turkeys. Poult Sci. 97: 2011-2020.
6. Kalmendal R, Tucson R (2012) Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. Poult Sci. 91: 1387-1393.
7. Masey O’Neill HV, Smith JA, Bedford MR. (2014) Multicarbohydrase Enzymes for Non-ruminants. Anim Biosci. 27: 290-301.
8. Menezes-Blackburn D, Gabler S, Greiner R (2015) Performance of Seven Commercial Phytases in an in vitro Simulation of Poultry Digestive Tract. J Agric Food Chem. 63: 6142-6149.
9. Vasquez MV, Glitsoe V. (2012) Phytase Unit Myth. https://www.dsm.com/content/dam/dsm/anh/en_US/documents/Phytase_unit_myths.pdf
10. Singh M, Krikorian AD. (1982) Inhibition of trypsin activity in vitro by phytate. J Agric Food Chem. 30:799-800.