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Development Of Feed Additives For Poultry Farming.

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ABSTRACT

This article presents the results of a study of the productivity of the fungus of the genus *Trichoderma* and the optimal nutrient medium based on soy processing waste to obtain a protein-enzyme feed additive. For the selection of the fungus genus Trichoderma, 3 species were used: *Trichoderma viride*, *Trichoderma lignorum* and *Trichoderma harsianum*. As a carrier for micromycete, 3 types of nutrient media were used, which were based on soybean Okara and additional sources of processing plant raw materials - sunflower husk, wheat husk (bran) and rice husk. As a result of the research, it was established that the *Trichoderma lignorum* mushroom on a nutrient medium, which is based on soybean okara and sunflower husk, shows the best results.

Keywords: nutrient medium, feed additive, fungus of the genus *Trichoderma*, enzymatic activity, crude protein, fiber, lignin, husk, soy okara.

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INTRODUCTION

According to the program “Development of Poultry Farming in the Russian Federation”, the volume of poultry meat production by 2020 should be increased to 9.5 million tons. This is expected to be achieved not only by increasing the range of poultry products, but also by expanding the food base with unconventional and at the same time cheap fodder means. As such agents, there are isolated preparations and enzymatic additives that increase the nutritional value of feed [1].

Enzyme preparations can increase the availability of fiber, protein and fat for the effects of enzymes of the digestive tract due to the destruction of the walls of plant cells; improve the digestibility of nutrients and their absorption in the small intestine; support intestinal microbiozin by reducing viscosity and increasing the level of simple carbohydrates; reduce feed costs for the increase in production; compensate for the lack of digestive enzymes in the early stages of development of young poultry and under stress, when the production of its own enzymes is limited [2, 3].

Recently, enzyme feed additives based on the fungus of the genus *Trichoderma* are of great interest, as it grows rapidly and produces various enzymes (cellulases, lignin dehydrogenases, xylanases, etc.), which makes it possible to use cost-effective components without sacrificing the nutritional value. ration. Due to the fact that the fungus of the genus *Trichoderma* is able to grow on almost any substrate, the use of soybean processing waste as the main carrier for solid-phase fermentation of the fungus with the aim of obtaining fodder enzyme additives is currently topical [4, 5].

In 2003, the Ministry of Agriculture of Russia adopted the sectoral program of the Russian Soybean Union “Development of the production and processing of soybeans in the Russian Federation for 2014–2020,” which confirms the relevance of using soybean waste. The implementation of the program has led to an increase in this crop in the country already to 740 thousand tons per year, and by 2020 it is planned to bring up to 12 million tons. According to literary data, within the framework of this program, 95% of soybean production waste is directed to processing them for feed purposes. Waste from soybean production, in particular, soybean okara contains a lot of fiber, protein, macro-, microelements and vitamins. The nutritional value of Okara is determined by the protein component, the complex of polyunsaturated fatty acids and oligosaccharides. Scientific studies have shown the presence of bifidogenic properties in soy oligosaccharides, which has a positive effect on the intestinal microflora.

The solution of these issues is consistent with the “Concept of state policy in the field of healthy nutrition of the population in the Russian Federation”, which places high demands on the balance of mixed feeds and rations that determine the quality of food products and, consequently, the health of the nation. In this regard, the board of the Ministry of Agriculture of the Russian Federation on November 29, 2011 made a decision (No. 15) on organizing the introduction of innovative technologies for feeding farm animals and poultry [6, 7, 8].

Thus, the use of soybean waste as a substrate for solid phase fermentation of the fungus of the genus *Trichoderma* in order to obtain protein-enzyme feed additives is a promising and relevant direction.

The aim of the research was the selection of the most productive type of fungus of the genus *Trichoderma* and the optimal nutrient medium based on waste from soybean to obtain a protein-enzyme feed additive.

MATERIAL AND METHODS

The work was carried out in the research laboratory of the Department of Biotechnology, Biochemistry and Biophysics FSBEI HE "Kuban GAU". For the selection of the fungus of the genus Trichoderma, 3 species were used: Trichoderma viride, Trichoderma lignorum and Trichoderma harsianum. As a carrier for micromycete, 3 types of nutrient media were used, which were based on soybean Okara and additional sources of processing plant raw materials - sunflower husk, wheat husk (bran) and rice husk. As indicators characterizing the efficiency of micromycete and substrate use, celluloseolytic activity was determined (GOST R 5304682008), protein content (GOST R 51417-99), lignin (GOST 26177-84), cellulose (GOST R 52839-2007) and reducing sugars (GOST 12575-2001) in the mixture [9, 10, 11, 12,].

The method for determining cellulolytic activity: three test tubes were filled with 1 cm^3 of the preparation, closed with stoppers and thermostated at 50 ° C for 5 minutes. In 2 test tubes made a solution of the enzyme preparation. All three tubes were kept at 50 ° C for 10 minutes. After carrying out the hydrolysis, 2 test tubes were added 3 cm^3 of the reagent DNS. The mixture was placed in a boiling bath for 5 minutes. Measured optical density on the PEC at a wavelength of 540 nm. To build the calibration curve, 2 cm^3 of standard glucose solution and 6 cm3 of potassium hexacyanoferote were added. The tubes were placed in a boiling bath for 10 minutes. Measured optical density at a wavelength of 440 nm. A graph was plotted: along the abscissa - molar concentrations of glucose, along the ordinate, optical densities. Cellulolytic activity was measured in U / g.

Method for determination of crude protein: 1 g of the additive was weighed into the tube with an error of 0.001 g. Insert the tube into the Kjeldahl flask to its bottom, pour out the hinge. Further carried out the mineralization. The mineralizer was transferred to a distant flask. The total volume of the solution in the distant flask is 200 cm^3. Next was made the distillation of ammonia to boric acid. The distillation flask was attached to the apparatus for the distillation of ammonia and, via a dropping funnel, a solution of sodium hydroxide was poured into the flask with the mineralized product. Stripping was performed with water vapor, in which the sulfuric acid is acidified to violet color using an indicator. At the beginning of the distillation solution is green. The end of the distillation set litmus test. The mass fraction of crude protein in the test sample was measured as a percentage.

Method for determining lignin: a weighed portion of 1 g with an accuracy of no more than 0.001 g was placed in a conical flask of 300 cm^3. 100 cm^3 of a 2% hydrochloric acid solution was added. The flask was closed with a rubber stopper with a glass tube inserted into it and boiled for 2 hours. The flask was removed from the tile and filtered through a funnel with a paper filter. The filter residue was washed with water until the acid reaction disappeared and then with acetone. The washed residue was hydrolyzed with a 72% solution of sulfuric acid: the residue from the filter was washed with acetone into the same conical flask, where the 2% hydrochloric acid solution was hydrolyzed. Acetone was evaporated in a water bath at 60 ° –70 ° C. 7 cm3 of a 72% solution of sulfuric acid was poured into the flask. The mixture was kept at 23 ° C for 2.5 hours. Then 93 cm^3 of water was poured in, closed with a rubber stopper with a glass tube inserted into it and boiled for 1 hour. The solution was filtered through an ashless filter placed on a Buchner funnel. The filter residue was thoroughly washed with acid in hot water. The filter with sediment is transferred to a buxu, dried for 4 hours at a temperature of 105 ° C, cooled and weighed. Then the filter with the sediment was placed in a porcelain crucible, kneaded in a muffle furnace (525 ° C) until constant weight was reached. The crucible with ash was cooled and weighed. The mass fraction of lignin was measured as a percentage.

Method for determination of fiber: a portion of 1 g was poured over 100 cm^3 of a 4% solution of sulfuric acid. Boiled for 10 minutes, and then the solution was sucked off with a Komovsky pump; for this, a Jandieri funnel with a paper filter was used. The funnel was carefully introduced into a glass before contact with the liquid and the solution was aspirated into a Bunsen flask. Then 28 cm^3 of potassium hydroxide solution was poured into a glass and boiled again for 10 minutes. The precipitate was transferred to a Buchner funnel with a paper filter, pre-dried and weighed with a weighing bottle. The residue in the bottle was dried in a drying cabinet, then cooled in a desiccator and weighed. Mass fraction of fiber was measured in percent.

Method for determination of reducing sugars: a portion of 10 g was transferred to a flask 200 ml using distilled water. A thermometer was put into the flask and placed in a water bath, heated for 15 minutes at a temperature of 80 ° C. 7 ml of a 30% solution of blue acetate was added to the flask, then 0.5 ml, then dropwise. Rapid exfoliation of clear liquid over the sediment indicates that lead acetate is sufficient. Then sodium phosphate was added in small portions. Distilled water was added to the flask to the mark and filtered through a folded filter. The filtrate (solution A) was poured into a burette. In 2 small conical flasks were pipetted into 10 ml of 1% solution of potassium ferricyanide and 2.5 ml each with 2.5 ml of 2.5 N KOH solution. The first flask was brought to a boil, 3 drops of 1% methylene blue solution were added. Not stopping the boil, titrated from the burette with solution A until the blue color disappeared. Titration in the second flask: 1 ml less solution A was added than during the first titration, then boiled for 1 min and 1 drop of methylene blue was added and titrated with solution A until the blue color disappeared. The content of reducing sugars was measured in percent [14].

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RESULTS AND DISCUSSION

The first series of experiments.

In the first series of experiments, we studied the primary indicators characterizing the efficiency of the use of the fungus and the nutrient medium: cellulolytic activity (GOST R 53046-2008) and protein content (GOST R 51417-99).

Cultivation of *Trichoderma viride* micromycete on various nutrient media.

The results of the solid phase fermentation of the fungus *Trichoderma viride* on various nutrient media are presented in table 1.

Table 1: Cultivation of the fungus *Trichoderma viride* on various nutrient media

Indicator	Soybean okara + sunflower husk	Soybean Okara + bran	Soybean Okara + rice husk
Cellulolytic activity, U / g	11,3	13,2	9,7
Raw protein,%	32,7	36,6	30,4

From table 1 it is seen that during the cultivation of the fungus *Trichoderma viride* on different nutrient media, we recorded the highest enzymatic activity (13.2 U / g) and the amount of crude protein (36.6%) on the medium containing soy-okara and wheat bran.

Cultivation of *Trichoderma lignorum* micromycete on various nutrient media.

The results of solid phase fermentation of the fungus *Trichoderma lignorum* on various nutrient media are presented in table 2.

Table 2: Cultivation of the fungus Trichoderma lignorum on various nutrient media

Indicator	Soybean okara + sunflower husk	Soybean Okara + bran	Soybean Okara + rice husk
Cellulolytic activity, U / g	31,6	27,3	23,4
Raw protein,%	42,8	37,2	35,8

The data in table 2 show that during solid-phase fermentation of the fungus *Trichoderma lignorum*, the highest enzymatic activity (31.6 U / g) and the amount of crude protein (42.8%) appeared on the medium containing soybean okara and sunflower husks.

Cultivation of *Trichoderma harsianum* micromycete on various nutrient media.

The results of the cultivation of the fungus *Trichoderma harsianum* on various nutrient media are presented in table 3.

Table 3: Cultivation of the fungus *Trichoderma harsianum* on various nutrient media

Indicator	Soybean okara + sunflower husk	Soybean Okara + bran	Soybean Okara + rice husk
Cellulolytic activity, U / g	14,3	10,2	10,7
Raw protein,%	32,1	31,7	30,3

From table 3 it can be seen that during solid-phase fermentation of the fungus *Trichoderma harsianum*, the highest cellulolytic activity (14.3 U / g) and the content of crude protein (32.1%) were found on the medium containing soybean okara and sunflower husk.

According to the studied parameters, it was concluded that the most productive micromycete is *Trichoderma lignorum*, grown on a nutrient medium containing soybean okara and sunflower husk.

The second series of experiments.

In the second series of experiments to study the effectiveness of the selection of the species of the fungus of the genus *Trichoderma* and nutrient medium, we studied the content of lignin (GOST 26177-84), fiber (GOST R 52839-2007) and reducing sugars (GOST 12575-2001).

Cultivation of micromycetes of the genus *Trichoderma* on various nutrient media according to the content of lignin, fiber and reducing sugars.

The results of the research are presented in table 4.

Table 4: Number of studied substances when growing micromycetes

Carrier	Indicator		
	Lignin, %	Cellulose, %	Reducing sugars, %
Trichoderma viride			
Okara + sunflower husk	33,1	29,3	15,1
Okara + bran	31,2	25,8	17,2
Okara + rice husk	35,4	31,9	14,2
Trichoderma lignorum			
Okara + sunflower husk	20,6	19,8	25,1
Okara + bran	23,5	22,8	21,3
Okara + rice husk	25,6	24,4	19,6
Trichoderma harsianum			
Okara + sunflower husk	32,1	27,5	16,7
Okara + bran	34,5	29,0	15,1
Okara + rice husk	29,6	26,2	18,2

As a result of the research, it was found that the best results were obtained, similar to the first series of experiments, when cultivating *Trichoderma lignorum* grown on a nutrient medium containing soybean okara and sunflower husks, since the amount of lignin and fiber in this mixture was lower than in other variants, and the amount of reducing sugars is higher, indicating a higher enzymatic properties that contribute to the destruction of complex carbohydrates into simpler monomers.

CONCLUSION

Microscopic fungus *Trichoderma lignorum* on a nutrient medium, which is based on soybean Okara and sunflower husks shows the best results. The resulting mixture can be used in poultry farming as a protein-enzyme feed additive to increase the digestibility of the feed, as well as the safety and productivity of poultry.

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