ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Optimization of Soy-Corn Substrate in the Cultivation of Microorganisms *Bacillus subtilis*.

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**ABSTRACT**

The article presents the results of studies on the optimization of components of probiotic feed additive made of soy-corn substrate for the active growth of microorganisms of the species Bacillussubtilis. Regression analysis of the experimental results showed that the maximum number of colony forming units (CFU)of microorganisms bacillussubtilis was obtained after cultivation in the substrate consisting of 220-270 millilitres of soy broth and 37-52 millilitres of corn broth. Probiotic feed additive is given to young farm animals to drink.

**Keywords:** soy, corn, Bacillussubtilis, colony forming units.

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ISSN: 0975-8585

## INTRODUCTION

Currently, biotechnology in animal husbandry is aimed at increasing the resistance of the animal organism, reducing the cost of livestock products and increasing the safety of feed[2,4].

Most probiotics contain microorganisms of the Bacillus species, which are used as a source of growth of organic substances. Microorganisms are resistant to the action of lytic and digestive enzymes and retain their life activity along the entire gastrointestinal tract. They are not toxic, so do not show pathogenic properties. Their antagonistic activity is actively expressed and manifests itself to a large range of pathogenic and conditionally pathogenic bacteria. The probiotic feed additive based on soy-corn substrate can satisfy the microbial needs of young farm animals in Bacillussubtilis [5].

## MATERIAL AND METHODS OF THE RESEARCH

The task of the research is to find a nutrient medium for the rapid growth of microorganisms Bacillussubtilis. The study is based on multivariate experiment [1]. The experimental results were processed by regression analysis and mathematical statistics. To construct the response surface and its sections, the program SigmaPlotv12 was used.

**Methods of preparation of broths**. Separately crushed, pre-soaked for 24 hours, 100 g of soy beans and 100 g of corn grains. The obtained masses were boiled separately in 1 litre of water for 20 minutes. After sterilization, the broths were mixed together and diluted with distilled water up to 500 millilitres. Active acidity was equalized to pH=7 using sterile buffer solutions. Microorganisms Bacillussubtilis in an amount of 2.5×107 colony forming units (CFU) were added to the obtained substrate. Cultivation of the microorganisms proceeded for 15 hours in the thermostat at the temperature of 37°C. The number of microorganisms was determined by the Koch method[3].

## RESULTS AND DISCUSSIONS

When analyzing the findings of the multivariate experiment, we can conclude that the maximum number of colony-forming units was in the 5$^{th}$ experiment: 200 ml of soy broth and 40 ml of corn broth (table 1).

**Table 1: results of the multivariate experiment**

| № | Factor Levels | | Findings of Investigation, CFU in 1 ml of the Product |
|---|---|---|---|
| | $x_1$ | $x_2$ | $\bar{y}_1$ |
| 1 | -1 | -1 | $5{,}70\times10^7$ |
| 2 | 0 | -1 | $1{,}91\times10^7$ |
| 3 | +1 | -1 | $7{,}83\times10^6$ |
| 4 | -1 | 0 | $1{,}36\times10^7$ |
| 5 | 0 | 0 | $5{,}85\times10^7$ |
| 6 | +1 | 0 | $4{,}78\times10^7$ |
| 7 | -1 | +1 | $4{,}22\times10^7$ |
| 8 | 0 | +1 | $4{,}57\times10^7$ |
| 9 | +1 | +1 | $4{,}31\times10^7$ |

After procession of the results of the experiment, an adequate regression equation was obtained. In the decoded form it took the following form:

$$CFU=751481389-7038054{,}5\times S-43605550{,}05\times C+422283{,}27\times S\times C+14330{,}55\times S^2+554138{,}82\times C^2-5278{,}54\times S\times C^2-859{,}83318\times S^2\times C+10{,}7479\times S^2\times C^2,$$

ISSN: 0975-8585

The construction of the response surface of the regression equation and its section was performed using a computer program SigmaPlotv.12 (figure 1,2).

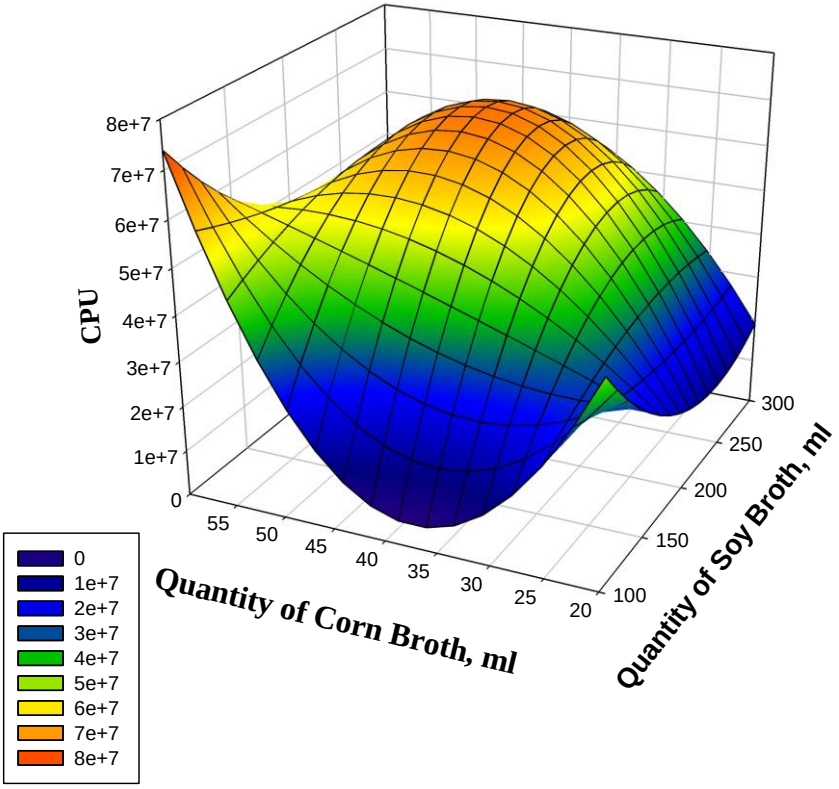


**Figure 1: The response surface of the number of colony forming units (CFU) depending on the amount of soy milk and corn broth**

When analyzing the response surface of CFU, we can conclude that the amount of soy and corn broth directly affect CFU. The most optimal amount of CFU is obtained when quantity of soybean broth varies from 220ml to 270 ml and 37-52 ml for corn broth. This is clearly shown in the section of the response surface (Fig. 2).

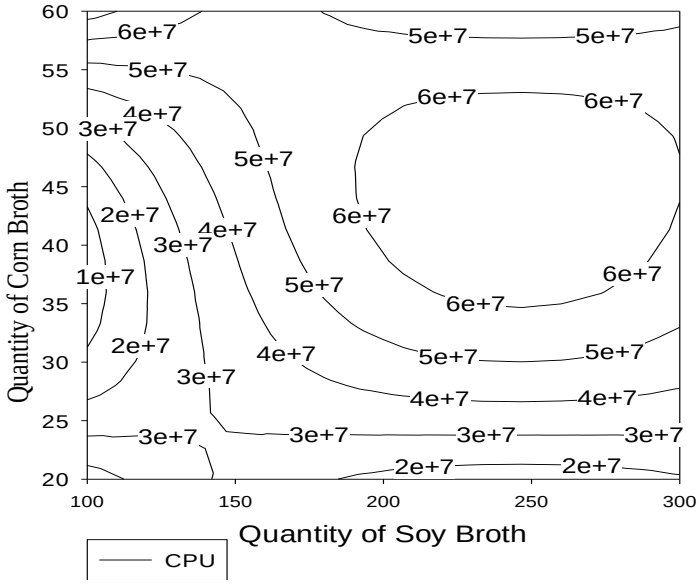


**Figure 2: Section of the response surface of the number of colony-forming units depending on the amount of soybean milk and corn broth.**

ISSN: 0975-8585

## CONCLUSIONS

As a result of the research, a probiotic feed additive was obtained, which allows of accumulation up to 6×107 CFU in 1 millilitre of the product with a nutritional value of 9.71-15.33 g of proteins and 13-14 g of carbohydrates, including 2.23-3.41 g of monosaccharides in 500 ml of the substrate, which is equivalent to whole milk.

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