Effect of probiotic cultures of the Bacillus amyloliquefaciens strain on the livability of broiler chickens

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Abstract. Under industrial conditions, broiler chickens are constantly exposed to stressors that can increase their susceptibility to intestinal diseases, leading to reduced productivity, immune suppression, and increased mortality. Antibiotics are widely used to maintain and improve productivity in such conditions. However, in recent years, there has been a tightening of rules on the use of antibiotics in animal husbandry in order to stop them from entering food for people, as well as in connection with the emergence of strains of pathogenic bacteria that have developed resistance to a wide range of antibiotics, which pose a danger to human health and life. In this regard, manufacturers are searching for alternatives to antibiotics. The range of potential substitutes is very wide. One of the most promising groups is probiotics. In the course of the study, it was found that low-protein diets (with a low protein content, but balanced by the amino acid profile) can unlock the potential of probiotic cultures of the Bacillus amyloliquefaciens strain and, as a result, increase the livability of broiler chickens. The maximum livability index of 94.5% was observed in group 4, which received a low-protein diet using probiotic cultures of the Bacillus amyloliquefaciens strain.

1. Introduction

Poultry farming in most countries of the world is still the largest source of full-fledged animal protein production. Poultry farming is an intensively developing and highly productive branch of animal husbandry, which provides the population with dietary food, and industry with raw materials. Science and practice have proved that poultry farming has the most favorable chances for rapid development and is able to make a significant contribution to ensuring the country’s food security in the next decade. In order for this industry to be competitive and profitable in a market economy, it must be based on highly productive livestock.

Industrial poultry farming in Russia makes a significant contribution to ensuring the country’s food security as the main producer of high-quality animal protein, the share of which in the daily diet of Russians reaches 40% due to the consumption of dietary eggs and poultry meat. The livability of poultry depends on the parameters of the microclimate, keeping technology, cross and age of the bird, but feeding has a decisive influence. Genetically modern poultry crosses have a high growth rate. In accordance with this, diets for it are developed so that the component composition provides the body with energy and nutrients.

Currently, the concept of poultry farms is based on reducing the time of fattening broilers in order to increase the profitability of production by increasing the turnover of the poultry herd per year.
For this purpose, the company’s specialists who feed poultry and farm animals pay great attention to the optimal composition of diets. Complete feed is made so that the energy and protein value is high for a rapid increase in live weight.

For broilers, full-fledged complete feeds are used, access to which is constant. Broilers should also have constant access to water.

Feed conversion is improved by creating new poultry crosses, reducing feed costs per unit of production, and using high-quality feed.

The level of animal protein should be 25% of the total amount in the complete feed, and if the latter is not enough, the norm of lysine and methionine is provided by synthetic amino acids. The humidity of dry food should not exceed 13%. At higher humidity they become compressed, stuck in bunkers, clog the feeders, disrupting the feeding regime. Microadditives should be dried and well mixed. The humidity of dry food should not exceed 13%. At higher humidity they become compressed, stuck in bunkers, clog the feeders, disrupting the feeding regime. Microadditives should be dried and well ground.

To eliminate the oxidation and destruction of vitamins, fortifiers from vitamins and trace elements are prepared separately.

At the same time, the high content of nutrients in the feed contributes to the strained functioning of the gastrointestinal tract, which leads to diseases of the digestive system. At the same time, the intestinal microbiocenosis is broken. The microflora of the gastrointestinal tract has colonial resistance, participating in the processes of digestion, providing immunity and detoxification. Rational use of protein by the poultry body depends on the balance of its amino acid composition and the level of availability of amino acids from complete feed. Protein is the basis for building the body and building up the live weight of broilers.

In modern poultry farming, it is a priority to find strategies for feeding broiler chickens that make the most effective use of the local raw material base, reduce the cost of nutrients for production and improve its quality. The effectiveness of feed use by broilers depends not only on the level of amino acids in them.

It is worth noting that it is almost always economically feasible to reduce the level of nutrients, while maintaining their balance, and increase the feed dose within a reasonable and possible range to meet the daily needs of poultry.

In the production of poultry products, a significant share of the cost is occupied by feed, and as we do not try, however, we can reduce this indicator without losing productivity only with great difficulty. The resulting reduction of each ruble in the calculation of huge volumes of production gives huge savings.

Currently, the main constraint on the further development of poultry farming is the limited feed resources. The cost of feed in the structure of the cost of poultry products is almost 60%. In this regard, an important area of research in the field of poultry feeding is the use of nutrients in the diet as fully as possible, which will reduce the share of cereals in diets.

The most important balancing element in feed is protein. Proteins are extremely complex organic compounds with a molecular weight measured in thousands, hundreds of thousands, and even millions of oxygen units (the weight of one oxygen unit is 1/16 of the weight of an oxygen atom). The main structural elements of a huge protein molecule are amino acids. They perform either independent functions, or participate in the construction of many biologically important compounds: purine and pyrimidine bases, nucleic acids, hormones, amines, peptides, etc.

Balancing poultry diets by amino acid composition is one of the most important aspects of effective and cost-effective feeding. In modern broiler crosses, the need for digestible amino acids is quite high.

In poultry, protein formation occurs under the action of enzymes and is directed by the genetic code that is embedded in nucleic acids.

About 20 amino acids were found in the composition of poultry body proteins. Various combinations of them cause a great variety of properties and quality of proteins. About half of these amino acids can be synthesized in the body of a bird. The formation of these, as well as other amino
acids that are not involved in protein synthesis, occurs as a result of restructuring the structure of carbohydrates, lipids, organic acids, amino acids, ammonium salts, etc.

Amino acids that can be synthesized in the body of a bird in an amount that provides the body needs for maintaining its normal existence and for the formation of products are called interchangeable. These amino acids include alanine, glutamic acid, aspartic acid, oxyproline, proline, serine, and glycine. It should be noted that the last amino acid is replaceable only for adult birds, but young animals are not able to synthesize it in the required amount.

Some amino acids can be synthesized by poultry if the body has sufficient amounts of other essential amino acids necessary for their formation. Such amino acids are referred to as partially interchangeable. This group includes cystine and tyrosine. Cystine can be obtained from methionine if there is enough of it in the body, and tyrosine from phenylalanine.

Therefore, the appropriate supply of poultry with cystine and tyrosine will depend on their intake in the body with food, as these two amino acids are essential. Thus, essential amino acids are not synthesized in the body or are synthesized in very small amounts that do not meet the need for them in poultry.

In addition to methionine and phenylalanine, essential amino acids for poultry include lysine, threonine, tryptophan, valine, leucine, isoleucine, arginine and histidine, and for young animals, glycine.

Lysine, which belongs to the group of essential limiting amino acids, is of particular importance in poultry feeding. In vegetable feeds, it is contained in small amounts, so animals often do not have enough of it, especially with the predominant use of cereals, sunflower meal and a small amount (1-2%) of animal feed.

In diets of wheat-barley and corn-sunflower type, lysine deficiency can reach from 15 to 20 percent. Therefore, synthetic lysine is widely used for their balancing, high levels of which in the form of monochlorohydrate lead to excessive content of chlorine in feed, causing its imbalance, which negatively affects the productivity of poultry. Currently, it is possible to achieve standard chlorine content by using lysine sulfate.

In industrial poultry farming, there is a downward trend in mixed feeds of soy meal as the main source of protein. This is due to its increasing cost and the use of genetically modified soybeans in cultivation.

Under industrial conditions, broiler chickens are constantly exposed to stressors that can increase their susceptibility to intestinal diseases, leading to reduced productivity, immune suppression, and increased mortality. One of the typical causes of intestinal microflora imbalance is an increase in the content of undigested nutrients in the intestinal lumen. Clostridium Perfringens is a bacterium that is usually present in the intestines of poultry in an amount below 105 EEC per gram of intestinal contents in the normal state (Caly et al., 2015) without adversely affecting the health of poultry. Clostridium Perfringens begins to show virulent properties when its population increases to 107-109 EEC per gram of intestinal contents (Kondo, 1988). The high level of feed protein content, in turn, is recognized as a factor contributing to the development of Clostridium Perfringens, and especially its strains capable of producing NetB-toxin, which is considered the main factor in the development of necrotic enteritis in broilers.

Antibiotics are widely used to maintain and improve productivity in such conditions. However, in recent years, there has been a tightening of rules on the use of antibiotics in animal husbandry in order to stop them from entering food for people, as well as in connection with the emergence of strains of pathogenic bacteria that have developed resistance to a wide range of antibiotics, which pose a danger to human health and life. In this regard, manufacturers are searching for alternatives to antibiotics. The range of potential substitutes is very wide. One of the most promising groups is probiotics. Probiotics were found to promote the development and maintenance of a stable gut microbiome in poultry, which leads to a reduction in the incidence of intestinal diseases and an increase in productivity. They can also improve feed conversion, reduce mortality, modulate the immune response, and protect against intestinal pathogens. The use of synthetic amino acids for balancing diets allows not only optimizing
the diet to reduce its cost without compromising production results, but also improving intestinal health, and therefore reducing the use of antibiotics for the treatment/prevention of intestinal diseases.

2. Methods
Scientifically-economic experience on the effect of probiotic cultures of the strain Bacillus amyloliquefaciens on the safety of broiler chickens was carried out on chickens of the cross Cobb-500 in terms of teaching and research poultry farm ESIC “Agricultural technical park” of Belgorod State Agricultural University in February-March 2020.

4 groups of 65 heads each were formed from a batch of chickens of one brood at the daily age. A total of 4 different diets were studied. The experiment lasted 40 days.

The parameters of the microclimate, planting density, feeding and watering front were similar for all groups of birds and corresponded to the standard indicators.

The bird received rations of the Starter, Growth, and Finisher brands. The Starter feed was received by the bird from the moment of setting for the experiment, on the 0th (1st) day. The transition from starter to Growth brand food was made at the age of 10 days by gradually replacing one feed with another (in % of the standard - 70/30, 50/50, 30/70). Then the bird was fed up to 22 days of age. From the age of 23 days, the bird was smoothly transferred to the Finisher feed (in the same proportion as when switching from Starter to Growth) and fed with this type of feed until the end of the experiment.

Features of feeding broiler chickens were as follows:
1 – Control group (Standard diet);
2 – Experimental group (Low protein diet);
3 - Experimental group (Standard diet + probiotic Bacillus amyloliquefaciens);
4 - Experimental group (Low protein diet + probiotic Bacillus amyloliquefaciens).

In experimental groups 2 and 4 (Low protein diet), the rations of GROWER and FINISHER additionally include the introduction of synthetic amino acids such as L-Valine, L-Isoleucine and L-arginine. The reduction in the whole protein content of feed in groups 2 and 4 will be about 1.5-2% for each phase. The reduction of the whole protein index in these groups is limited by balancing the so-called “glycine equivalent” (the sum of glycine + serine x 0.7143), where the calculated value of this indicator is at least 118% of the total lysine.

During the experiment, the following indicators were determined:
- microclimate data in the poultry house (daily) (temperature, humidity, ventilation parameters in m³/h per head, illumination);
- feed distribution accounting (daily);
- accounting for diseases/waste/mortality (daily);

The conditions for keeping chickens in all groups were the same and met the recommended standards for this cross

3. Results
Numerous studies have found that the conditions of keeping poultry often serve as stress factors that reduce the efficiency of agricultural production.

Data on the microclimate of broiler chickens are presented in table 1.

| Day | Temperature, °C | Humidity, % | Ventilation, m³/h per head | Illumination, Lux |
|-----|----------------|-------------|-----------------------------|-------------------|
|     | Min | Max | 66 | 0.06 | 25 |
| 1   | 34.6 | 35.0 | 66 | 0.06 | 25 |
| 2   | 34.0 | 34.5 | 66 | 0.09 | 25 |
| 3   | 33.3 | 33.9 | 68 | 0.12 | 25 |
The body reacts to the influence of environmental factors depending on its adaptive capabilities. The specificity of adaptive reactions depends on the initial functional state, the period of adaptation, etc.

Table 2. Livability of broiler chickens, %.

| Group       | Livability of broiler chickens by groups, % |
|-------------|--------------------------------------------|
| 1 group (Control) | 90.3                                       |
| 2 group (1 experimental) | 89.9                                       |
| 3 group (2 experimental) | 94.0                                       |

| Group       | Livability of broiler chickens by groups, % |
|-------------|--------------------------------------------|
| 4           | 33.4                                       |
| 5           | 32.8                                       |
| 6           | 32.4                                       |
| 7           | 31.7                                       |
| 8           | 30.9                                       |
| 9           | 31.5                                       |
| 10          | 29.9                                       |
| 11          | 29.3                                       |
| 12          | 28.8                                       |
| 13          | 28.9                                       |
| 14          | 27.6                                       |
| 15          | 28.7                                       |
| 16          | 27.9                                       |
| 17          | 28.8                                       |
| 18          | 26.0                                       |
| 19-21       | 26.9                                       |
| 22-24       | 25.6                                       |
| 25-27       | 25.1                                       |
| 28-36       | 21.2                                       |
| 38          | 20.6                                       |
| 39          | 20.2                                       |
| 40          | 19.8                                       |

| Group       | Livability of broiler chickens by groups, % |
|-------------|--------------------------------------------|
| 5           | 33.9                                       |
| 6           | 32.8                                       |
| 7           | 32.3                                       |
| 8           | 31.3                                       |
| 9           | 32.1                                       |
| 10          | 30.5                                       |
| 11          | 29.9                                       |
| 12          | 29.2                                       |
| 13          | 29.5                                       |
| 14          | 28.0                                       |
| 15          | 29.3                                       |
| 16          | 28.3                                       |
| 17          | 29.4                                       |
| 18          | 26.6                                       |
| 19-21       | 27.9                                       |
| 22-24       | 27.4                                       |
| 25-27       | 26.1                                       |
| 28-36       | 25.6                                       |
| 38          | 21.2                                       |
| 39          | 20.8                                       |
| 40          | 20.6                                       |
During the experimental period (from day to 40-day age), the physiological state of the bird was monitored daily. In order to determine the effect of the use of probiotic cultures of the Bacillus amyloliquefaciens strain on the resistance of the bird body, we evaluated its safety for individual growing periods and in general for the entire period of the experiment (table 2).

Livability of chickens demonstrates that the lowest figures recorded in the groups not receiving the feed probiotic, where there was safety level 89.9-90.3 %. The maximum survival rate 94.5% was observed in the 4th group receiving low protein diets with the probiotic cultures of the Bacillus amyloliquefaciens strain.

4. Summary
The range of potential substitutes for antibiotics is very wide. One of the most promising groups is probiotics. Probiotics have been found to promote the development and maintenance of a stable gut microbiome in poultry, which leads to a reduction in the incidence of intestinal diseases and an increase in productivity.

Probiotics improve feed conversion, reduce mortality, modulate the immune response, and protect against intestinal pathogens.

The use of synthetic amino acids for balancing diets allows not only optimizing the diet to reduce its cost without compromising production results, but also improving intestinal health, and therefore reducing the use of antibiotics for the treatment (prevention) of intestinal diseases.

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