Screening for Reducing Ammonia Emissions in Broiler Feed Probiotics

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Abstract: The purpose of this study was to screen Probiotic can improve feed utilization rate, improve broiler gut environment, reduce emissions of ammonia in the purpose of broiler manure. Through the simulation of the digestive tract of broiler environment, high yield cellulase, amylase, protease, screening of microbial inhibition of gut harmful bacteria, and conduct the feeding experiment. The results show that the concentration of ammonia in the chicken house when feeding Bacillus subtilis for 58 hours was 13.814 mg / m³, which was lower than that of the People's Republic of China Agricultural Industry Standard NY / T 1167-2006 Environmental Quality Standard for Livestock and Poultry Farms, Experiments show feeding broilers with Bacillus subtilis can reduce the production of harmful gas ammonia and effectively improve the environment of livestock and poultry houses.

1. Introduction

Broilers have a short intestinal tract, and the nutrients in the feed, such as proteins, amino acids, and nucleic acids, are not completely digested and absorbed, and harmful gases that affect broiler health are formed after fermentation in the body or in vitro. The most harmful to broilers is ammonia. Ammonia mainly comes from incompletely digested feed residues, which are formed by decomposition of nitrogen-containing organic matter. This is related to the protein content in the feed and the digestibility of the chicken [1]. The high concentration of ammonia in the chicken house directly stimulates the mucous membrane of the chicken, including the respiratory mucosa, conjunctiva, and cornea. Continued increase in irritation will cause broiler keratitis, conjunctivitis, dermatitis, bursal atrophy, air sac inflammation, and Escherichia coli disease [2], reducing broiler production performance.

At present, there are two main types of deodorant research in domestic and foreign chicken houses, one is in vitro deodorant and the other is in vivo regulator [3]. In vitro deodorants include masking deodorants, chemical deodorants and adsorbents. In vivo regulators, especially feed deodorants in the form of probiotic preparations, have been receiving continuous attention in recent years. Adding deodorant as feed can reduce the production of harmful gas components in the excreta, reduce the pollution of the chicken house environment from the source, and improve the health level and production performance of livestock and poultry.
2. Materials and method

2.1. Materials

2.1.1. Instruments
Ultraviolet spectrophotometer; clean bench; biochemical incubator; automatic Kjeldahl nitrogen analyzer; portable livestock and poultry house environment detector, etc.

2.1.2. Reagents
Phosphate buffer (pH6.5), phosphate buffer (pH2.0), artificial gastric juice, artificial intestinal juice, beef extract peptone, YEPD, Chass medium, MRS medium, protease screening plate, cellulose-Congo red Isolation medium, NH3-determinating bacteria screening medium [4], solid starch medium. NH3 detection kit.

2.1.3. Strain
This laboratory preserves broiler feeding bacteria: *Bacillus licheniformis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Lactobacillus plantarum*, *Candida utilis*, *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae*, *Bacillus coagulans*.

2.2. Method

2.2.1. Screening of beneficial bacteria

2.2.1.1. Preliminary screening of tolerant probiotics in intestines of broilers
Take appropriate amount of probiotics cultivated to the end of the logarithmic phase respectively, and mix the bacterial mud with artificial gastric juice after centrifugation. Put it in a 42 °C constant temperature water bath for 40 minutes, centrifuge again to take bacterial mud and artificial intestinal juice and mix, and put it in a 42 °C water bath to keep warm. Take appropriate amount of mixed liquid at heat preservation 0h, 2.5h, 6h, 12h, 24h, calculate the number of viable bacteria by plate colony counting method to determine the strain of intestinal tolerance in broilers.

Because it is considered that increasing feed utilization and reducing intestinal free ammonia content can reduce ammonia emissions from broilers, the following studies were conducted on the selected resistant strains.

2.2.1.2. Screening for effective use of free NH3 strains
The candidate strains were spot-connected to a nitrogen-free screening plate and cultured at a suitable temperature. If grown, it showed the ability to utilize NH3.

2.2.1.3. Using high yield protease, amylase and cellulase as double screening conditions
Screening of high-yield protease-producing strains: The candidate strains are spot-connected on a casein plate screening plate and cultured at a suitable temperature, and a transparent circle is observed. Enzyme production capacity is determined by comparing the size of the transparent circle.

Screening of high-yield amylase strains: Connect the candidate strains to the starch hydrolysis screening plate. After culturing at a suitable temperature, add an appropriate amount of iodine solution to the plate and observe whether a transparent circle appears. If it appears, it indicates that amylase is produced. Enzyme production capacity is determined by comparing the size of the transparent circle.

Screening of high-yield cellulase strains: as a screening plate. The strains were spotted on a cellulose-Congo red screening plate and cultured at a suitable temperature to observe whether a transparent circle appeared, and if it appeared, it indicated that cellulase was produced. Enzyme production capacity is determined by comparing the size of the transparent circle.
2.2.2. Initial feeding test to determine ammonia content in feces

Through the above screening test, the excellent strains were determined and the feeding test was conducted. Ammonium nitrogen in the form of ammonia or ammonium ions in the feces reacts with the Nessler reagent, and the color of the product is proportional to the content of the ammonium nitrogen. It can be determined spectrophotometrically[5], combined with the NH₃ detection kit to determine Ammonia content.

A total of 36 four-month-old roosters were selected. The control group was set up with 6 animals in each group, a total of 1 group, fed with basic diets. There are 6 animals in each group in the experimental group, a total of 5 groups. Feed the probiotics to be tested into the feed for feeding, with a cycle of five days. In the test, a feces receiving bottle was installed at the anus of each chicken. Fresh chicken manure was subjected to gradient dilution to determine the ammonia content.

2.2.3. Test the feeding again to determine the ammonia content in the house

The excellent probiotic strains screened in the initial feeding results were fed again, and the ammonia content in the chicken house was measured by a portable livestock and poultry house environmental detector, with a view to screening for the best deodorant probiotic bacteria.

There are 5 experimental groups identified, each with 20 adult males, and the experimental period is 3 days. In order to eliminate the interference of non-test factors, fasting (water only) for 58 hours before each test, and then feeding for 3 consecutive days, feeding twice per day according to the standard of feeding 100g per chicken. The bacterial solution and feed are mixed in a ratio of 1: 2. Clean the manure once every test cycle.

3. Result and Analysis

3.1. Probiotic screening test results

3.1.1. Preliminary screening test results of tolerant microorganisms in intestines of broilers

By counting the live bacteria on the plate, the results are shown in Table 1:

| Strain                        | Total number of colonies cfu/g |
|-------------------------------|--------------------------------|
|                               | 0h    | 2.5h   | 6h    | 12h   | 24h   |
| Saccharomyces cerevisiae      | 4.0×10⁶ | 3.0×10⁴ | 2.0×10⁴ | 1.9×10⁴ | 2.0×10⁴ |
| Saccharomyces cerevisiae      | 6.5×10⁵ | 3.2×10² | 2.1×10² | 1.8×10¹ | 51    |
| Candida utilis                | 2.0×10⁶ | 1.8×10³ | 9.5×10³ | 3.5×10⁵ | 2.2×10⁶ |
| Lactobacillus plantarum       | 2.1×10⁷ | 5      | 4      | 3      | 3     |
| Bacillus subtilis             | 3.0×10⁷ | 4.5×10³ | 2.9×10³ | 1.4×10³ | 1.4×10³ |
| Bacillus licheniformis        | 3.5×10⁸ | 6.3×10³ | 2.7×10³ | 2.0×10³ | 2.4×10³ |
| Enterococcus faecalis         | 6.2×10⁷ | 5.4×10⁵ | 3.6×10⁵ | 2.7×10⁴ | 2.8×10⁴ |
| Bacillus coagulans            | 5.0×10⁷ | 4.5×10³ | 3.0×10³ | 3.2×10³ | 4.5×10³ |

The results showed that under the same culture conditions, Lactobacillus plantarum and Saccharomyces cerevisiae died quickly in broilers. The reason may be that they were sensitive to pH and could not tolerate the intestinal environment of broilers. The residual amount of cells in 24 hours was almost negligible Not counted, so it can be eliminated directly. Saccharomyces cerevisiae, Candida utilis, Bacillus subtilis, Enterococcus faecalis, Bacillus licheniformis and Bacillus coagulans can still reach 104-105 in 24 hours, so they are determined as intestinal tolerant strains.
3.1.2. Screening results for effective use of NH3 strains
Saccharomyces cerevisiae, Candida utilis, Bacillus subtilis, Enterococcus faecalis, Bacillus licheniformis, and Bacillus coagulans were spotted on a denitrification-free screening medium plate. Yeast, Bacillus subtilis, Enterococcus faecalis, Bacillus coagulans, and Bacillus licheniformis can all grow.

3.1.3. Test results with high yield protease, amylase and cellulase as double screening conditions
The protease-producing strains are Candida utilis, Enterococcus faecalis, Bacillus licheniformis, and Bacillus subtilis.

The strains with high amylase production capacity are in order Enterococcus faecalis, Bacillus coagulans, Bacillus licheniformis, and Bacillus subtilis.

The strains with high cellulase production capacity are Enterococcus faecalis, Bacillus coagulans, Bacillus licheniformis, and Bacillus subtilis.

3.2. Results of the initial feeding test
Based on the above screening tests, the excellent strains for improving feed utilization and reducing intestinal free ammonia content were identified as Candida utilis, Enterococcus faecalis, Bacillus licheniformis, and Bacillus subtilis, and the feeding test was conducted.

Since the ability of Candida utilis to produce protease is particularly outstanding, the Candida utilis was combined with the above three bacteria to investigate the symbiosis between the strains. At the same time, conduct feeding test. However, the results of the bacterial feeding test are shown in Table 2:

Table 2. After single bacteria feeding the determination results of NH3 concentration in waste

| Strain                  | Ammonia content (mg/l) |
|-------------------------|------------------------|
| Blank control           | 8.04                   |
| Bacillus coagulans      | 5.45                   |
| Enterococcus            | 4.83                   |
| Bacillus subtilis       | 3.73                   |
| Candida utilis          | 4.13                   |

The results show that: compared with the blank, the four single bacteria have a certain ability to degrade NH3 in feces. Among them, Bacillus subtilis has the best effect of reducing NH3. The ammonia concentration in feces is only 3.73mg / l, followed by Candida utilis only 4.13mg / l.

The results of the dual-bacteria combination test are shown in Table 3:

Table 3. After feeding double bacteria combination probiotics the determination results of NH3 concentration in waste

| Strain                              | Ammonia content (mg/l) |
|-------------------------------------|------------------------|
| Blank control                       | 8.04                   |
| Candida utilis and Enterococcus     | 6.25                   |
| Candida utilis and Bacillus coagulans| 2.85                   |
| Candida utilis and Bacillus subtilis| 5.62                   |
| Candida utilis and Bacillus licheniformis| 5.22                |

The results showed that compared with the blank, the combination of Candida utilis and Bacillus coagulans had the best effect of reducing NH3, and the ammonia content in feces was only 0.285mg / l.

3.3. Feeding test results again
In order to screen the probiotic bacteria with the best ammonia deodorization effect, the ammonia content in the chicken house was measured by a portable livestock and poultry house environmental
detector. The combination of fecal cocci, Candida utilis and Bacillus coagulans was fed again, and the results are shown in Figure 1:

![Figure 1. Line chart of changes in the concentration of ammonia after feeding](image)

From the analysis of Figure 1, it can be seen that according to the change of ammonia content, compared with the blank group, the four test subjects all have a certain effect of reducing ammonia emissions. The effect of ammonia reduction in the closed chicken house is always the most obvious. The ammonia concentration of the chicken house was 13.814 mg/m³ when feeding Bacillus subtilis for 58 hours, and the concentration of ammonia was 15.848 mg/m³ for 58 hours when the combination of Candida utilis and Bacillus coagulans was fed.

The People's Republic of China Agricultural Industry Standard NY/T 1167-2006 Livestock and Poultry Farm Environmental Quality Standard[8] The NH₃ concentration in the chicken house should not be higher than 15 mg/m³. According to research[6, 7], in order not to affect the performance of chickens, the NH₃ concentration in the house should be less than 20 mg/m³. Therefore, this study determined that Bacillus subtilis can effectively reduce the production of harmful gas after feeding broilers, which is conducive to the improvement of the environment of livestock and poultry houses and meets the requirements of national industry standards.

4. Results
This study started with the selection of artificial simulated gastrointestinal fluids and bile salt-tolerant strains, through the selection of high-protease, amylase and cellulase strains, and the ability to degrade free NH₃. By comparing the feeding trials of various combinations of single and double bacteria, the fresh manure of broilers and the ammonia content in the house were determined. The test results showed that the mass concentration of ammonia in the chicken house was 13.814 mg/m³ when feeding Bacillus subtilis for 58 hours, which was lower than the environmental quality standard of the livestock industry of NY/T 1167-2006 of the People's Republic of China. Feeding broilers can reduce the production of harmful gas ammonia and effectively improve the environment of livestock and poultry houses.

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