Effects of Probiotics on Breeding Environment and Intestinal Microorganisms of Livestock and Poultry

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Abstract [Objectives] The paper was to study the effects of probiotics on breeding environment of livestock and poultry and intestinal microorganisms of breeding animals. [Method] Chicken houses and hog houses were selected as the research objects. The breeding house supplemented with basal diet was used as the control group; the breeding house sprayed with probiotics product 1 was experimental group 1 (exogenous treatment); the basal diet mixed with probiotics product 2 and drinking water mixed with product 3 was experimental group 2 (endogenous treatment); and the combination of the two experimental groups was experimental group 3. [Results] Experimental groups 1 and 2 reduced the concentration of ammonia in hog houses and chicken houses, and the effect of experimental group 2 was better than that of experimental group 1. Experimental group 3 had the best effect, which significantly reduced the ammonia concentration in hog houses and chicken houses by 28.67% and 34.09%, respectively, and the average ammonia concentration outside the house within the range of 100–300 m was less than 2 mg/m³. Moreover, it significantly increased the number of intestinal lactobacillus and reduced the number of Escherichia coli. [Conclusions] Probiotics can effectively reduce the ammonia concentration in livestock and poultry breeding environment, and improve the intestinal microbial structure of breeding animals. The technology of spraying exogenous probiotics combined with the use of content-derived probiotics has great potential for popularization and application.

Keywords Probiotics; Livestock and poultry; Breeding environment; Intestinal microorganisms

uring the 14th Five-year Plan period, the domestic food healthy concept and the demand for highquality livestock and poultry products continues to increase, and the requirements on the specifications and quantity of modern livestock and poultry industry are enhanced. Meanwhile, the accompanying volume of dung from livestock and poultry breeding will also increase significantly. For example, the feces output from livestock and poultry breeding in China increased rapidly from 1.9 billion t in 1999 to 4 billion t in 2010^[1]. However, pollution-free treatment of feces is less than 10%[1], and more than 80% of breeding feces are stored off-site^[2]. Data show that ammonia emissions from China's breeding industry account for about 60% of the total amount of ammonia in China^[3], which significantly increases the pressure

on the breeding environment and directly and indirectly affects the healthy development of the breeding industry. With the development of deodorization technology for breeding environment, deodorization technology based on probiotics has entered the vision of scientists and breeding technicians, becoming a research focus of improving the breeding environment in China^[4]. Through literature search, it was found that the current domestic research on the application of probiotics in the improvement of breeding environment mainly focused on exogenous treatment of probiotics spraying[5] and endogenous treatment of probiotics mixed with diet or drinking water^[6]. However, few efforts have been dedicated to systematic studies on exogenous and endogenous treatment of probiotics, especially the lack of data support for practical application of such probiotics. Therefore, this paper studied the effects of probiotics on the livestock and poultry breeding environment and the intestinal microorganisms of breeding animals through two kinds of treatment methods of probiotics, so as to provide the theoretical reference for further promotion and application of these products in animal husbandry.

1 Materials and Methods

1.1 Animals and materials White-feathered broilers and commercial pigs were selected as experimental animals. White-feathered broilers were 4-6 weeks old and pigs were at fattening stage. Three kinds of probiotics were used in livestock and poultry experiments, all of which were derived from Ningde Enterprise Technology Center of Fujian Beidi Pharmaceutical Co., Ltd. Probiotics product 1 was bacillus liquid preparation (viable count ≥1.0×10⁸ CFU/mL); product 2 was fermented solid preparation (viable count ≥1.0×10⁸ CFU/g); product 3 was lactobacillus liquid prepa-

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ration (viable count $\geq 1.0 \times 10^8$ CFU/mL).

1.2 Experimental design and feeding management The experiment was conducted in a chicken farm and a pig farm in Zherong County. Four breeding houses were randomly selected, with 1 000 whitefeathered broilers in each chicken house and 100 pigs in each hog house. The chicken houses and hog houses were under the same feeding conditions and feeding and drinking conditions. The experimental animals were healthy and had normal appetite. Four groups were designed in the test. The breeding house supplemented with normal basal diet was used as the control group (CK); the breeding house sprayed with probiotics product 1 on breeding house site and fecal surface was experimental group 1 (A); the basal diet mixed with probiotics product 2 and drinking water mixed with product 3 was experimental group 2 (B); and the combination of the two experimental groups was experimental group 3 (C). According to the preliminary experimental data, product 1 in group A was diluted with water till its concentration became 10% of the original value and sprayed by a sprayer every morning on the ground, walls and air; product 2 in group B was mixed with the diet according to 5% for broiler and 6% for pig and product 3 was added with water at the dose of 5%. The diets of whitefeathered broilers and pigs were provided by breeding farms. Dietary feeding, drinking water management and sanitation management methods were consistent. The experiment lasted for 7 d.

1.3 Experimental indexes and analysis methods

1.3.1 Ammonia concentration in the house. Ammonia concentration in the chicken house and hog house was detected by fixed-point sampling and concentration determination with ammonia detector. Ammonia sampling points were located in the front, center and back of the chicken house and hog house, respectively, and the height of sampling points was 1.0–1.2 m

above the ground. The ammonia concentration in the house was sampled and measured at the end of the 7^{th} day of the experiment, and the data were recorded. The concentration unit was mg/m³.

1.3.2 Ammonia concentration outside the house. The experimental group 3 (C) of chicken house and hog house was selected to detect the ammonia concentration outside the breeding house at the end of the 7th day of experiment. The ammonia sampling point was at the prevailing wind direction of the breeding house, and the sampling point of ammonia concentration outside the breeding house was 100, 200 and 300 m outwards respectively with the breeding house as the center point. The ammonia detection equipment and methods were the same as described above, and the data were recorded. The concentration unit was mg/m³.

1.3.3 Number of intestinal microorganisms. At the end of the 7th day of the experiment, 10 broilers were randomly captured in each group of experimental groups C and CK. The cecal contents of broilers were removed under aseptic operating conditions and loaded into aseptic cryopreservation tubes. Under the aseptic operation of cryopreservation tube, 0.5 g of each caecal content was taken and mixed in a sterile test tube containing 5 mL of normal saline, and then diluted step by step. The diluent was poured onto lactobacillus medium plate (MRS) and eosin methylene blue (EMB) plate, respectively, and incubated at 37 °C for 48 h. The number of colonies in the plate was counted by colony counting method, and the viable count of lactobacillus and E. coli in cecal contents of broilers was calculated. Similarly, 9 copies of fresh pig feces were randomly collected from each group in the front, center and back of pig houses in experimental groups C and CK, respectively, and the fecal samples were mixed and diluted step by step under aseptic operation. The diluent was incubated by the above-mentioned two kinds

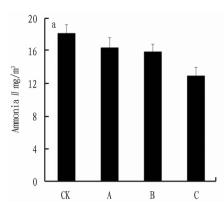
of media, and the viable count of lactobacillus and *E. coli* in pig feces was calculated.

1.4 Statistical analysis All data were expressed by "mean \pm standard deviation". The data were done one-way analysis of variance (ANOVA) by IBM SPSS Statistics 25 software, and performed multiple comparison analysis by LSD method (Duncan analisis). P < 0.05 indicated significant difference.

2 Results and Analysis

2.1 Effect of probiotics on ammonia concentration in the breeding house As shown in Fig.1, the ammonia concentrations in the breeding house in experimental groups A, B and C were significantly reduced compared with blank control CK (P<0.05). In hog houses, the ammonia concentrations in experimental group A and B were lower than that in CK at the end of the 7th day, but there was no significant difference between the two groups, while the ammonia concentration in experimental group C was extremely significantly reduced (P<0.01, Fig.1-a). In chicken houses, the ability of reducing ammonia concentration in the house in experimental groups A, B and C significantly increased successively (P < 0.05), and group C could significantly reduce the ammonia concentration in the house (P< 0.01, Fig.1-b). The results showed that exogenous spraying of probiotics and endogenous feeding and drinking probiotics both had the effect of reducing ammonia concentration, and the combined use of exogenous and endogenous probiotics received enhanced effect.

2.2 Effect of probiotics on ammonia concentration outside the breeding house Based on the influence of different experimental groups on the ammonia concentration in the breeding house, experimental group C, namely the combined use of exogenous and endogenous probiotics, had the best effect on reducing the am-



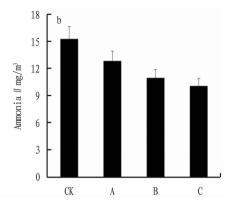


Fig.1 Effects of different experimental groups on ammonia concentration in breeding houses

monia concentration in the breeding house. In order to study the effect of experimental group C on the ammonia concentration outside the breeding house, the ammonia concentration outside the breeding house was detected. As shown in Fig. 2, experimental group C had consistent but varying degrees of influence on the ammonia concentration outside the breeding house of pigs and broilers. The average ammonia concentration outside the house was about 1.67 mg/m³ within the range of 100 –300 m, and the ammonia concentration outside the hog house and chicken house was lower than that of CK

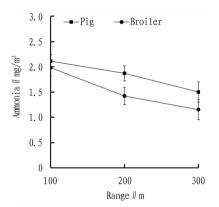


Fig.2 Effect of experimental group on ammonia concentration outside breeding houses

group, and the concentration decreased with the increase of distance.

2.3 Effects of probiotics on intestinal microbe of breeding animals As shown in Tab. 1, the combine use of exogenous and endogenous probiotics in experimental group C significantly affected and improved the intestinal microbiological parameters of breeding animals compared with blank control CK. Compared with CK, experimental group C significantly increased the number of lactobacillus in cecal contents of broilers and pig feces (P < 0.05), and significantly reduced the viable count of E. coli (P<0.01). The results showed that the combine use of exogenous and endogenous probiotics could improve the viable count in the intestinal microbial structure of breeding animals, and was conducive to the cultivation of beneficial intestinal probiotics and the inhibition of harmful intestinal bacteria.

3 Discussion

Probiotics have become one of the research hotspots in improving the breeding environment in China. The strains involved mainly are *Bacillus subtilis*, lactobacillus and actinomycetes. Meantime,

Tab. 1 Effects of experimental group on intestinal microbiological indexes of broilers and pigs ${
m CFU/g}$

Group	Broiler		Pig	
	Lactobacillus	$Escherichia\ coli$	Lactobacillus	Escherichia coli
Blank control	7.12±0.26	7.23±0.33	7.52±0.32	7.14±0.26
Experimental group	7.98±0.20*	6.31±0.29**	8.11±0.21*	5.98±0.46**

Note: *represents significant difference compared with blank control group (P < 0.05); **represents extremely significant difference compared with blank control group (P < 0.01).

exogenous treatment of spraying probiotics and endogenous treatment of probiotics mixed with diet or drinking water is the major application mode of probiotics. Traditional exogenous spraying treatment has certain effect on breeding environment space and contact surface, while endogenous treatment of probiotics mixed with diet or drinking water can improve the intestinal flora and metabolites of intestinal contents and feed utilization rate of breeding animals by feeding microecological bacteria and metabolites^[7], thus further reducing the excretion of ammonia in animal feces. The effects of endogenous and exogenous treatments of microecological agents on the livestock and poultry breeding environment and intestinal microorganisms of breeding animals were studied, and data analysis results showed that the two treatments had different effects on ammonia concentration in livestock and poultry breeding houses. Endogenous treatment combined with exogenous treatment was superior to endogenous treatment, and then superior to exogenous treatment, and the reduction of ammonia concentration in the breeding house directly affected the ammonia concentration in the environment outside the house. Therefore, the combined emission reduction technology of endogenous ammonia and exogenous ammonia is more suitable for modern livestock and poultry breeding. At the same time, statistical analysis of the data in this study showed that the number of intestinal microorganisms in breeding animals was positively correlated with the change of ammonia concentration, and the higher the ratio of lactobacillus and E. coli, the more conducive to ammonia emission reduction, which provided a new idea for the further indicator research of our team.

4 Conclusions

The results showed that the ammonia concentration in hog houses and chicken houses could be reduced by spraying exogenous probiotics, but the effect was worse than that of the endogenous experimental group of probiotics mixed with diet or drinking water. Meanwhile, the combined use of exogenous probiotics with content-derived probiotics had the best effect, which could significantly reduce the ammonia concentration in hog houses and chicken houses by 28.67% and 34.09%, respectively, and the average ammonia concentration outside the house within the range of 100-300 m was lower than 2 mg/m³. Moreover, it significantly increased the number of lactobacillus and reduced the number of E. coli, and improved the intestinal microbial structure of breeding animals.

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