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RESEARCH ARTICLE

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Effects of microbial agents on small intestinal structure and the quantity of cecal microorganisms in broilers

Abstract In order to elucidate the significance and related mechanisms of microbial agents in modulating the growth of broilers, one-day-old Avian broilers were employed to investigate the effect of microbial agents on body weight, daily gain and feed efficiency at various stages. The birds in the experimental groups were given salmonella pullorum then fed with antibiotics to counteract it. The results showed that the average broiler weight of the oral microbial agent group at the age of 40 days was significantly increased by 16.58% from 1520 g to 1772 g ($P < 0.05$). The feed conversion efficiency was decreased from 2.21 to 1.82 ($P < 0.01$). In the experimental group fed with microbial agents, microscopic observation revealed that the intestinal villi increased in number, length and orderliness. The intestinal wall became thickened. The glands in the intestine were developed, which would be beneficial to nutrient absorption. The effect of oral microbial agents was identical to that due to antibiotics. It was also observed that *Lactobacillus* dominated the cecal microorganisms in broilers.

Keywords microbial agent, broilers, intestinal structure, cecal microorganisms

1 Introduction

With the development of the modern poultry farming industry, the direct contact between chicks and their parents is often blocked, which makes it impossible for the chicks to get the rapid vertical transmission of intestinal microbia from their parents, leading to the temporal lapse in the establishment of normal intestinal microbial flora in chicks, thus affecting nutrient conversion. It was reported that in the commercial broiler farming industry in the years 2000 to 2001, the broiler's body weight reached 2.10 kg at 35-days of age and feed-conversion ratio was 1.5∶1 (Dou, 1998), through the application of more and more feed additives such as weight gain promoters and antibiotics, which highlighted the problem of drug residues and drug-resistance of the microorganisms. These problems severely affected the export of fowl products. With the impact of the international market, China's broiler industry has been encountering a very hard situation, and the demands of quarantine on meat products including broilers are increasingly getting stronger in most countries. According to international standards, tranquilizers in muscle, liver and kidney should not exceed 20 $\mu g \cdot kg^{-1}$ (Xue, 1996) and neither should piperazine and butyrophenone. More and more broiler products cannot reach such standards. Therefore, according to the principle of balance of animal intestinal microflora, our present study systematically investigated the effect of microbial agents, which are used to replace antibiotics, on the performance and immunological function of intestinal microflora, intestinal structure and nutrient utilization rate in broilers. Our study was to provide a practical basis for developing feed additives and their proper utilization in the poultry industry. As a naturally used bioactive agent, a microbial agent could overcome many of the shortcomings of antibiotics, and it is non-toxic and has no side-effects. Microbial agents have many advantages over antibiotics or other growth-promotion agents. Microbial agents produce healthy and green meat products with great economic and social benefits.

Although many other reports have confirmed that feeding microbial agents could promote growth and increase body weight, what they do is just add certain microbial agents, such as lactobacillus, bacillus, photosynthetic bacterium or compound bacterium, etc. into feeds (Yi et al., 2004). In order to verify the effectiveness of applying microbial agents in broiler production, the intestinal contents of healthy broilers were directly collected and cultured in liquid medium, which was

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taken as the microbial agent in our study, containing enough microbes to balance the intestinal microbial flora in the new-born chicks. The experiment was designed to understand the mechanisms of its promoted body weight gain, enhanced feed conversion rate and improved immune function by analyzing the changes in the quantity of cecal microorganisms or the intestinal villi length before and after feeding microbial agents.

2 Materials and methods

2.1 Preparation of microbial agent

Cecal content samples were aseptically collected from 200 SPF adult chickens and then suspended in normal saline at 1:10. The samples which tested negative for salmonella, haemolyticus colibacillus, and coccus were used in the test. The suspension was cultivated for 48 h with the thermostat at 32°C in liquid medium, which was laid at 121°C for 15 min first, cooled before inoculation, centrifuged (5000×g) for 20 min, and thereafter the bacterial cells were collected by humic acid adsorption, with a free humic acid content of 31% and nitrogen content of 4%, then mixed to get the products used in this experiment, which contained microbes at 5×10^8 CFU·g^{-1}, and stored at 4°C until use.

2.2 Experimental animal

Two hundred one-day-old Avian broilers were randomly divided into four groups, with 50 broilers in each group raised in one cage (480 cm×80 cm×120 cm). Cage temperature was controlled at 30°C to 32°C with a thermometer placed at 5 cm above the cage floor. Environmental temperature (room temperature) was 20–25°C and the relative humidity was maintained at around 30%.

2.3 Salmonella velogenic strain

The salmonella velogenic strains were provided by the Biological Institute of Hebei. Cat No. 80-552. Each broiler received a gavage of 40 million colony forming units from broth culture.

2.4 Experimental vaccine

Triple attenuated Vaccine VH-28/86-120 was purchased from The Biological Products Company, Beijing.

2.5 Ration composition

The ration composition in the experiment was divided into two phases, 1–4 weeks were the first prophase, 5–8 weeks was the second stage. The rations for the two stages were prepared by the authors themselves.

2.6 Experimental design

One-day-old chicks in Group 1 and Group 2 were gavaged with 0.5 mL mixed bacilli solution daily (including microbes 10^9 CFU·g^{-1}), consecutively for seven days. Chicks in Group 2 and Group 3 were challenged with salmonella at the 7th day. Birds in Group 3 were supplemented with norfloxacin into their diet (50 mg·kg^{-1}) the next day. Group 4 was kept as control. All the chicks were vaccinated with VH-28/86-120 triple attenuated vaccine by intranasal droppings at the age of 8 days.

2.7 Growth experiment

Body weight and feed conversion ratio in each group was detected at the age of 40 days. Their health status, the quantity of dead broilers, the mortality and survival rate were also monitored.

2.8 Histological observation of the micro-structure of the small intestine in broilers

Five chicks in each group were selected randomly for micro-observation before the termination of experiment. Small intestine samples were fixed in Bouin's solution for 24 h then dehydrated and paraffin-embedded. Five micrometer sections were prepared and stained by H&E. The structures of the small intestine were observed under a light microscope.

2.9 Cecal microorganism count

Five 50–day-old chickens in each group were selected randomly for micro-observation. Samples of cecal contents were taken under sterilized conditions, and placed in anaerobic diluent, gently shaken while being diluted gradually. The samples were then placed into Broth medium for microecological analysis. The lactobacilli, total anaerobic bacterium, and *E. coli* were counted. The data were analyzed by the SAS (1989).

3 Results

3.1 Changes in broiler performance

Oral application of microbial agents significantly augmented the weight gain in Group 1 compared to that of Group 4 ($P < 0.01$). There was also a significant difference in feed conversion ratio between Group 1 and Group 4 ($P < 0.01$) (Table 1).

Table 1 Body weight gain and feed-conversion ratio in different groups

group	body weight(g) at age 40 d	feed-meat rate at age 40 d
Group 1	1772 ± 68^{a}	1.82 ± 0.16^{a}
Group 2	1550 ± 73^{bc}	1.85 ± 0.13^{bc}
Group 3	1440 ± 45^{b}	1.92 ± 0.26^{b}
Group 4	1520 ± 55^{c}	2.21 ± 0.25^{c}

Note: The different small letters indicate $P<0.05$. Same in the following Tables.

3.2 Observation of the intestinal structures

The microstructures of the small intestinal tissue sections showed that, compared with the control group, intestinal villi in Group 1 increased greatly in number and grew thicker, longer, and more orderly in arrangement. The intestinal wall became thicker, with developed glands which would increase the contact area and facilitate secretion of digestive enzymes and absorption of nutrients. In Group 2, the birds were given microbial agents and then challenged with salmonella, therefore the small intestinal villi became thinner and longer. Since Group 3 was treated with antibiotics after the salmonella attack, the intestinal villi became disorderly in arrangement, the quantity was reduced, the intestinal wall was thin, and intestinal glands were undeveloped, which reduced broilers' feed intake resulting in retardation of the growth rate. From the thickness of the intestinal wall and the length of the intestinal villus in each group in Table 2, we can see that the intestinal villus length and thickness of the intestinal wall in Group 1 were higher than those of the control group, with a significant difference ($P<0.01$). But as the second group was supplied with microbial agents and attacked with narcotics, there was no significant difference ($P>0.05$). Similarly when Group 3 and Group 4 were treated with no microbial agents, the intestinal wall thickness and length of the intestinal villi were so similar that there was no significant difference ($P>0.05$).

Table 2 Intestinal wall thickness and length of intestinal villus in each group

group	length of the intestine villi/μm	intestinal wall thickness/μm
Group 1	1229.6 ± 271.2^{A}	375 ± 38.6^{a}
Group 2	1032.2 ± 204.5^{AB}	318 ± 47.5^{ab}
Group 3	564.3 ± 161.6^{C}	156 ± 66.3^{b}
Group 4	721.5 ± 132.0^{C}	282 ± 52.8^{b}

Note: The different small letters indicate $P<0.05$, capital letters indicate $P<0.01$.

3.3 Changes in the quantity of lactobacilli, *E. coli*, anaerobic bacterium in caecum

Table 3 shows that the quantity of cecal anaerobic bacteria in Group A was significantly higher than that of the control Group C, with a significant difference between them ($P<0.05$), but no significant difference with Group B (Norfloxacin) was found ($P>0.05$). The quantity of cecal *E. coli* in Group B was slightly higher than that in Group A and Group C. The numbers of lactobacilli in Group A and Group B were on average higher than that in Group C, with a significant difference ($P<0.01$). As chickens were fed with microbial agents, their *in vivo* antibiotic drugs were reduced, resulting not only in chicken growth, but also in enhancement of meat quality because of the large amount of beneficial microorganism activation *in vivo*. From the quantity of cecal microorganism in experimental chickens, we could see that adding microbial agents in chicken diet will reduce the quantity of cecal *E. coli* and increase the quantity of cecal beneficial lactobacilli.

Table 3 Changes of the quantity of lactobacilli, *E. coli*, anaerobic bacterium in caecum

microorganism	microbial agents A	norfloxacin B	control C	SEM
lactobacil$\times10^{8}$	7.98^{a}	8.7^{ab}	3.79^{b}	1.60
anaerobic bacterium$\times10^{9}$	7.95^{a}	5.47^{ab}	2.10^{b}	32
E. coli$\times10^{8}$	1.20	2.1	1.40	0.54

4 Discussion

The growth of the digestive organs of broilers during their lifetime of 7 weeks mainly occurs before the age of four weeks (Zhang, 2000; Zhang, 2002). Therefore, good absorption of enough nutrients at that time point is vital to ensure the rapid growth of the digestive system in broilers (Gu et al., 1993). Healthy visceral organs could enhance the anti-stress capacity of broilers (Fang et al., 1999). A favourable digestive system could guarantee rapid growth of the broilers. Microbial agents as additives are very important for stimulating the appetite and facilitating digestion and absorption in broilers. In the present study, at the later growth stage of the broilers (40 days old), the effects of adding microbial agents on the growth rate and feed conversion rate were significant, for instance' oral application of microbial agents had a more significant difference ($P<0.05$) in Group 1 than in Group 3 in terms of feed conversion ratio from 2.21 in Group 1 to 1.82 in Group 3. There was no significant difference between Group 2 and Group3 ($P>0.05$) because many microbes in the microbial agents could produce amylase, lipase, protease and other digestion enzymes, which can degrade starch, protein and other macromolecules to a certain degree, thereby transforming into monosaccharides, glucose and amino acids, and other small molecules which are more easy to digest and absorb for poultry. Intestinal acidification is beneficial to the absorption of Fe, calcium and vitamin D. Zhang et al. (1999) reported that adding microbial agents at a chicken's early stage could significantly increase the activity of some digestive enzymes in the serum and intestinal tract, such as amylase, protease and lipase enzymes, especially after the age of 20 days. Adding microbial agents at 0–0.6% daily could increase the early growth rate of the broiler and feed conversion rates. Meanwhile, the growth and reproduction of intestinal beneficial microorganisms could produce all kinds of nutrients including vitamins, amino acids, unknown agents, etc. that participate in the metabolism of the body, which has a satisfactory effect in promoting early growth and improving feed conversion rates in broilers.

In addition, as microbial agents are able to adjust the micro-ecological balance of poultry's endosomatic microbes with different functions that could grow and metabolize productively *in vivo*, in turn facilitating the absorption of various feedstuff in chicks and reducing unnecessary energy consumption, they thereby improve the feed conversion rate (Gao et al., 2002) and lower the feed-meat rate.

The small intestine is the main place of nutrient absorption. Firstly, liquid can easily pass through the very thin inner surface of intestinal mural epithelium mucosae into the blood stream; Secondly, the intestine is long enough for nutrients to have a tremendous absorption surface; Thirdly, there are millions of villi on the intestine's epithelial mucosae to further expand the absorption surface. Villi are extremely dense in the duodenum and jejunum, with a gradual reduction in the ileum. Around each villose periphery there is a single band of columnar epithelial cells, the epithelial cells which have special absorptive capacity. They dispose several hundreds of digitations (microvilli) at the edge of the epithelial enteric cavity, about 1–1.5 μm length, and 0.1 μm width, so that the absorption area can be increased by several hundred folds. After the nutrients are absorbed into the villi, they go into the systemic circulation by lymph and blood. Due to these special structures, a substantial increase in the length of the intestinal villus will increase the absorption area of the small intestine, making more nutrients in the diet be fully absorbed and utilized, which can promote the growth of animals, elevate the average weight in 40 days and improve the feed utilization rate. Uauay (1990) added nucleotides into the ration of weanling rat of 2 weeks. The results showed that the height of mouse intestinal villi, bowel wall thickness, total protein quality, and DNA quantity were higher than those of the control group. It proved that nucleotides can accelerate the differentiation, growth and repair of enterocytes. In this experiment, through microscopic observation of small intestine tissue sections in 40–day-old broilers, we found that groups treated with microbial agents improved the small intestinal structure, the small intestinal villi were longer and more regular in arrangement than those of the control group, and the epithelial mucous membrane of the small intestine was thinner. This change was more conducive for digestion and absorption of nutrients in the small intestine. The result that microbial agents improved the length of intestinal villi was consistent with the results of Choi. The increase in the intestinal villi could enlarge the contact surface of enteric chyme with epithelial cells, therefore it promoted enteric absorption capacity. The experiments by Sataka showed that different doses of microbial agents could stimulate intestinal epithelial cell proliferation.

There are a lot of microorganisms in a normal animal intestinal tract. Tian et al. (1999) pointed out that at a general state of health, the dominant intestinal populations are anaerobic bacteria in pigs or chickens (99% or more), and aerobic and facultative anaerobes (only 1%), such as bacteroid, bifidobacterium and lactobacillus, etc. But when animals are in a stringent state, facultative anaerobic bacilli increase significantly, and anaerobic bacilli are reduced significantly. This may induce diarrhea or dysentery, but the dominant microbial population was restored when a microbial agent was added into the animals in their normal condition. Through this experiment, the improvement of performance on broilers treated with microbial agents was attributed to the role of lactobacilli (Yu, 1990). It also agrees with the conclusion that the lactobacillus averages in the experimental groups and the control group were significantly different ($P < 0.1$). Studies have found that lactic acid generated by lactobacilli during their growth is transformed from *Veillinlla alcaleascens* into volatile fatty acids (VFA), which decrease the intestinal pH value. VFA has antibacterial and disinfectant capacities and inhibits the growth and reproduction of pathogenic bacteria in the intestine. Meanwhile, lactobacillus can produce lactic acid peptides to protect the intestinal mucosa from being invaded by other pathogenic organisms, therefore, it reduces colonization by harmful bacteria such as salmonella and *E. coli* in the cecum, and stabilizes the viable organism balance (Ren et al., 1999).

Accordingly, we have drawn the conclusion that *E. coli* was decreased more significantly in the experimental groups compared with the control group. The increase of lactobacillus was due to the additive microbial agent taken from the normal bacteria flora of healthy animals. The microbial agent can enable the chickens to establish normal bacteria flora rapidly which can produce a variety of useful microorganisms (Li et al., 2001) through their autospecific propagation and metabolism. The anaerobes also increased obviously, and 99% of the normal intestinal flora was anaerobic bacteria. It is the protective layer on the intestinal mucous membrane surface formed by these normal intestinal flora that resists the invading pathogens and enhances the disease resistance of the broilers.

5 Conclusions

Oral application of a microbial agent in broilers can make the intestinal walls become thicker and the intestinal villi longer and orderly arranged. The agent can also increase the surface area of the small intestine, which is in favor of digestion and absorption of nutrients. Meanwhile, the quantity of cecal microbiota and the total quantity of anaerobic bacteria in broilers can also be enhanced by the microbial agent. Through clinical observation, the application of a microbial agent can improve the disease resistance and performance of broilers and reduce the death rate. This has obvious economic and social benefits and a prosperous future.

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