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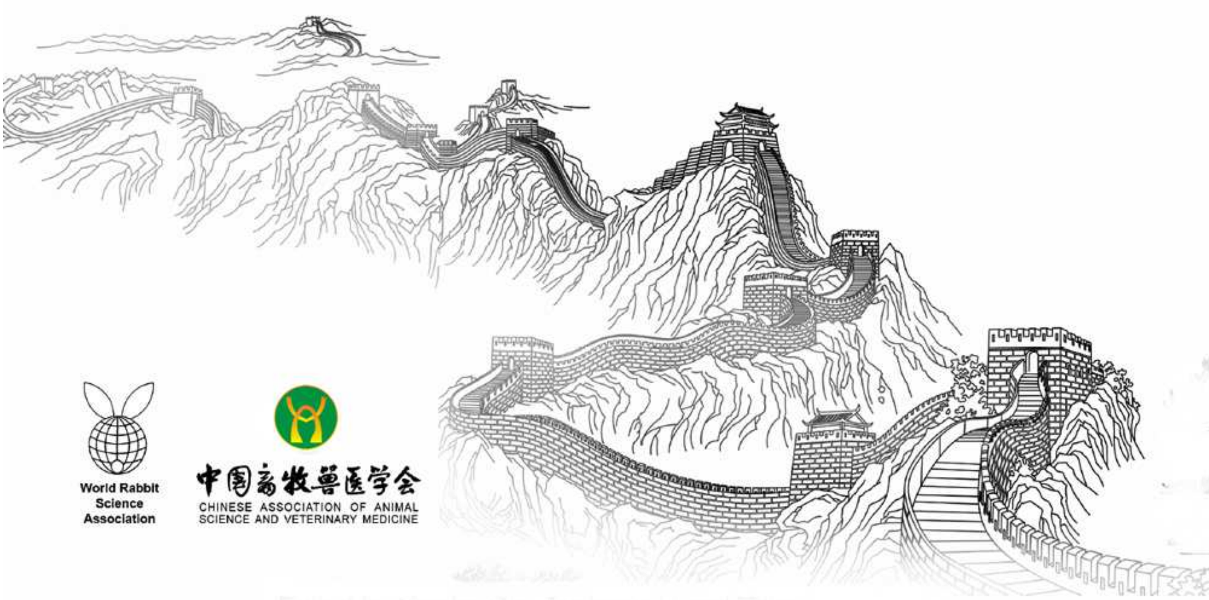

EFFECTS OF *BACILLUS COAGULANS* ON PERFORMANCE AND INTESTINAL PHYSIOLOGY OF GROWING RABBITS

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ABSTRACT

The effects of dietary *Bacillus coagulans* on growth performance, intestine pH, caecal microflora were evaluated in the growing rabbits. The trial used 150 New Zealand rabbits, weaned at 35 days divided into 3 groups, with 5 replicates per groups and 10 rabbits per replicate. The rabbits in control group were fed with basal diet without any probiotic， while those in experimental groups were fed the basal diet supplemented with 250 and 300 mg/kg *B. coagulans* respectively． The experiment lasted for 8 weeks. The two supplemental levels of *B. coagulans* increased by 20% the daily gain and feed conversion ratio ($P < 0.05$). It also reduced the pH in duodenum, jejunum, ileum and caecal contents, and reduced the number of total aerobic bacteria and E.coli in caecum ($P < 0.05$), but increased the number of anaerobic bacteria. The results suggested that supplementation of *B. coagulans* would increase the growth performance of rabbits, by regulating the pH of intestinal and thus perfecting the gut microbiota. The appropriate amount of *B. coagulans* for rabbits was 250 to 300 mg/kg under our experimental conditions.

Key words: *Bacillus coagulans*, rabbits, growth performance, probiotic

INTRODUCTION

Feeds with antibiotics resulted in many problems, such as drug resistance, drug residues and eco-environmental damage and so on (Mellon *et al.*, 2001). Developing the more efficient, non-toxic side effects, no residue and safety of new feed products has becoming one of the most important problems to solve the intestinal health of your rabbits. *Bacillus coagulans* shows high resistance to heat and capability of preservation. As a new functional feed additive, the effects of *B. coagulans* as probiotic has been confirmed in humans, aquaculture, swine, and poultry. But it's effect in rabbit has not been studied. This study aimed to investigate the effect of *B. coagulans* as probiotic in rabbit diet. The production performance and intestinal physiology, including of intestine pH and caecal microflora, were used as indicators.

MATERIALS AND METHODS

Animals and experimental design

Bacillus coagulans used in this study is provided by TU Biotechnology Limited Guangzhou Green, product approval number: (2014) 158 121, the count of viable bacterium $\geq 2.0\times 10^{10}$ CFU/g. New Zealand Rabbits were provided by the Sichuan animal science academy. According to single factor experiment design, 150 of 35-day-old weaned rabbits were randomly divided into three groups, with 5 replicates per groups and 10 rabbits per replicate (male and female for each 1/2). The rabbits were raised in individual cages with natural illumination. The experiment lasted for 8 weeks. Control group was fed basal diet, the treatment groups were fed the diets adding *B. coagulans* at the concentration of 250mg / kg (T-1) and 300mg / kg (T-2). Ingredients of basal diet (pelleted) was: alfalfa meal 30.0%, corn 21.0%, soybean meal 12.7%, wheat bran 21.0%, wheat 7.0%,

rapeseed meal 4.0%, $CaHPO_4$ 0.9%, limestone 0.7%, NaCl 0.5%, 98%Lysine 0.2%, premix 1.0%. The nutrient calculated levels were: digestible 10.41MJ/kg (as fed), crude protein 16.2%, crude fibre 13.1%, calcium 1.0%, Phosphorus 0.6%.
At 84d old, 10 rabbits in each group were slaughtered and 5 g of contents of duodenum, jejunum, ileum and cecum were sampled. The pH value of contents were measured immediately (3 replicates). Cecal chymes were sampled and the number of *E. colibacillus*, *Lactobacillus*, *Bifidobacillus*, total aerobic bacteria, and total anaerobes bacteria were measured by plate cultivation. The bacteria numbers were calculated by log base 10.

Statistical analysis
Data were analysed by the one-way analysis of variance and Duncan's method. Probability levels of 0.05 were used to determine the significance in all treatments. Results were expressed in "mean + / - standard deviation". All statistical analysis of data was performed using SPSS14.0.

RESULTS AND DISCUSSION

Effects of *B. coagulans* on growth performance of the rabbit
Initial weight, final weight, ADG, ADFI and F/G of New Zealand rabbits given diets containing different probiotic levels were shown in Table 1. There was no significant difference in ADFI (P>0.05) among control and experiment groups. The lowest ADG was found in control group (P<0.05) and there were no significant differences between the two other groups. The highest F/G was observed in the control group, higher than the experimental groups (P<0.05). The test group 1 was 4.10, lower than the test group 2 (P< 0.05).

Table 1 Effects of *B. coagulans* on growth performance of growing rabbits

Item	Control group	T-1	T-2
ADFI/(g/d)	101±3.6	112±4.8	112±6.1
ADG/(g/d)	$23.5±0.3^{a}$	$27.3±0.3^{b}$	$28.9±0.4^{b}$
F/G/(g/g)	$4.29±0.07^{c}$	$4.10±0.05^{b}$	$3.89±0.03^{a}$

In the same row, values with different small letter superscripts mean significant difference(P<0.05),while with the same or no letter superscripts mean no significant difference (P>0.05).The same as below.

Effects of *B. coagulans* on intestinal pH of growing rabbits
For all intestinal pH, probiotic supplementation showed a decreasing trend compared with the control (P < 0.05, Table 2). However, there was no significant difference between groups T1 and T2 (P > 0.05).

Table 2 Effects of *B. coagulans* on intestinal pH of growing rabbits

Items	Control group	T- 1	T- 2
Duodenum	$6.18±0.25^{b}$	$5.95±0.21^{a}$	$5.88±0.19^{a}$
Jejunum	$6.92±0.13^{b}$	$6.57±0.11^{a}$	$6.52±0.11^{a}$
Ileum	$6.89±0.16^{b}$	$6.37±0.13^{a}$	$6.32±0.12^{a}$
Caecum	$6.04±0.13^{b}$	$5.42±0.10^{a}$	$5.42±0.09^{a}$

Effects of Bacillus cereus on intestinal microflora of meat rabbits
The number of *E. coli* of control group was higher than those of groups T1 and T2 (P <0.05, Table 3), the lowest number was founded in T-2 (P <0.05). For *Lactobacillus*, the lowest was in the control group compared with experiment groups (P <0.05), the highest was T-2, but there was no difference between the T1 and T2 groups (P> 0.05). The same result was observed for *Bifidobacteria*. Compared to the control group, the total aerobic bacteria of experimental groups were reduced (P <0.05). For total anaerobic bacteria, the lowest was observed in the control group, which lower than experiment groups (P <0.05), and between the T1 and T2 groups there was no difference (P> 0.05).

According to the lower F/G for T1 and T2 groups, this suggested that the young rabbit improved the utilization of nutrients. The result was consistent with Rengpipa's reports that *Bacillus* can promote the weight of shrimp（Rengpipat *et al.*, 1998. *Bacillus* itself has the function of acid production, and also by degrading the fibre material, and glycolysis way to produce more volatile fatty acids. So, the animal's intestinal pH is reduced. Our results showed that the *Bacillus* would rebuild acid environment of rabbit duodenum, jejunum, ileum and caecum（Padiha *et al.*, 1995. Compared with other livestock, rabbit's caecum takes up an important position in the digestive system（Haresing *et al.*, 1977), especially the microflora plays a very important role in the process of digestion, absorption of nutrients, as well with disease prevention. So, the stability of caecum microflora becomes a main problem to maintain the rabbit health. Adamia report showed that Bacillus addition to the diet for suckling pig can colonize the intestines, and would inhibit the growth of E. coli（Adamia *et al.*, 1999. By studying the effect of *Bacillus* to the performance of weaned piglets, Hyronimus reported that Bacillus can produce coagulin during metabolism, which inhibits the growth of *Enterococcu*s, *Leuconostoc*, *Listeria* etc. Our results presented similar effects with other livestock and poultry（Hyronimus *et al.*, 1998).

Table 3. Effects of *B. coagulans* on intestinal microflora of the growing rabbits (log CFU/g)

	Control group	T-1	T-2
E. colibacillus	$5.21{\pm}0.20^{c}$	$4.73{\pm}0.15^{b}$	$4.21{\pm}0.11^{a}$
Lactobacillus	$5.77{\pm}0.25^{a}$	$6.86{\pm}0.29^{b}$	$7.09{\pm}0.30^{b}$
Bifidobacillus	$5.63{\pm}0.23^{a}$	$6.05{\pm}0.25^{b}$	$6.09{\pm}0.29^{b}$
Total aerobic bacteria	$7.01{\pm}0.34^{b}$	$6.39{\pm}0.28^{a}$	$6.16{\pm}0.24^{a}$
Total anaerobes bacteria	$8.81{\pm}0.37^{a}$	$9.39{\pm}0.41^{b}$	$9.44{\pm}0.43^{b}$

CONCLUSIONS

Under the test condition, the appropriate adding amount of *B coagulans* would 250mg/kg~300 mg/kg for growing rabbit feed.

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