IMPACT OF PROBIOTICS ON INTESTINAL MICROBIAL COMMUNITY DIVERSITY OF GROWING REX RABBITS

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ABSTRACT

An experiment was conducted to determine the effects of *Lactobacillus* on growth performance, serum metabolite, the number of mast cells and microbial community diversity of growing Rex Rabbits. A total of 120 rabbits weaned at age of 30 days were randomly divided into four groups. The control group was fed on basal diet only. The group I was fed on basal diet with antibiotics (*Zinc Bacitracin*), and group II and III were fed on basal diet adding *Lactobacillus zeae* (LB1) and *Lactobacillus rhamnosus* (L3) respectively. The results obtained were as follows: feed to gain ratio (F/G) of the rabbits fed with *Lactobacillus* isolates was significantly lower than that of control group ($P<0.05$). The diarrheal incidence from group I to III was 20%, 23.3% and 30% respectively which was significantly lower than that of control group (36.7%). The concentration of alanine amiotransferase (ALT) was significantly lower ($P<0.05$) when rabbits were fed with *Lactobacillus* isolates LB1 and L3. Meanwhile, the concentration of IgG and IgM increased significantly ($P<0.05$). *Lactobacillus* isolates had no influence on the number of mast cells in jejunum and duodenum ($P > 0.05$) but increased the number of mast cells in caecum significantly ($P<0.05$). The analysis of Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) indicated that *Lactobacillus* isolates can adjust the intestinal microbial community diversity of growing rabbits. In conclusion, the application of *Lactobacillus* isolates LB1 and L3 all can increase the growth performance, enhance the immunologic function and adjust the intestinal microecosystem of growing Rex Rabbits.

Key words: Rex Rabbit, *Lactobacillus*, microbial community diversity, intestine

INTRODUCTION

The rabbit is kind of typical herbivores whose diet contains large amounts of cellulose which is decomposed by intestine microorganisms. So the gastrointestinal tract of rabbit is a complex ecosystem that often harbors a diverse bacterial community which has become an integral component of the host and may affect the host biology (Ley, 2008). An important reason for high diarrhea morbidity and mortality of young rabbit was disorder of intestine bacteria. Antibiotics may kill symbiotic microorganisms; however ill-use of antibiotics may kill helpful bacterial and disrupt the balance of intestine ecosystem of rabbits.

Probiotic bacteria are defined as living microorganisms that exert beneficial effects on animal health (Naidu, 1999). *Lactobacilli are* the most commonly used probiotic bacteria (Ljungh, 2006). *Lactobacilli* have been found to have a variety of physiological influences on their hosts, including antimicrobial effects, microbial interference, supplementary effects on nutrition, antitumor effects, reduction of serum cholesterol and lipids, and immuno-modulatory effects (Wang, 2011; Collado,2009). However, there have been few studies concerning the influence and mechanism of *Lactobacilli* on rabbits breeding, especially for intestine biodiversity of Rex Rabbits.

In this paper, we aimed to study the influence of *Lactobacillus zeae* (LB1) and *Lactobacillus rhamnosus* (L3) on Rex Rabbits, including growth performance, immunologic function, and microbial diversity of intestine.

MATERIALS AND METHODS

Bacteria culture

Lactobacillus isolates: *L. zeae*(LB1) and *L.casei* (L3). Either de Man Rogosa Sharpe (MRS) broth or agar was used to culture *Lactobacillus* isolates at 37℃ for 18 to 24 hrs in an anaerobic chamber with an atmosphere of (85% N_2, 10% CO_2, and 5% H_2), the number of the bacteria was greater than 10^9CFU/mL.

Animal and diets

A total of 120 Rex Rabbit weaned at the age of 30 days (629±39g, P > 0.05) were allotted to 4 groups on the basis of weight and gender in a randomized complete block design. There were 30 rabbits in each group and the rabbits were housed individually in wire cages. All rabbits had free access to water and feed during the trial. The control group only fed on basal diet. The composition and nutrient level of basal diet were shown in Table 1. Group I fed on the basal diet with antibiotics (*Zinc Bacitracin*, 100mg/kg mixed in basal diet). Group II and III fed on the basal diet adding 0.3% (v/v, 3ml *Lactobacillus* liquid has been culture described as before per Liter water) *Lactobacillus* isolate LB1 and L3 in water respectively.

The experiment lasted for 35d, including a 7d adjustment period and a 28-d experimental period. Individual weights were measured at the beginning (day 37) and the end (day 65) of the experiment, and the average daily gain (ADG) was calculated. The average daily intake (ADI) was recorded and feed to gain (F/G) ratio was calculated. The numbers of weaned rabbits with diarrhea was recorded everyday and diarrheal incidence was calculated accordingly.

On day 65, 40 rabbits (10 rabbits per group, male and female having half each, with their body weights around the average group body weight) were bled by cardiac puncture for blood samples and then were killed by exsanguination from the carotid artery. The blood samples were centrifuged at 1,500 × g for 10 min. The isolated serum samples were stored at -20°C for further analysis. The content of intestine were collected respectively and frozen immediately in liquid nitrogen and subsequently stored at -70°C before DNA extraction. Meanwhile, the jejunum, duodenum and caecum were cutted at same station and washed using 0.9% NaCl respectively, which were mobilized in Bouin′s solution for further experiment (Li, 2006).

Table 1: Ingredients and composition of the experimental diets for the growing rabbits (as fed-basis).

Ingredients %	Percent (%)	Nutrients	Computed result
Maize	21	Crude protein	16.001
Soybean meal	18	Crude fat	2.153
Wheat bran	21	Crude fiber	14.53
CaHCO3	1.5	Digestible energy	10.106
NaCl	0.5	Calcium (Ca)	0.632
Corn germ meal	10	Total phosphorus (TP)	0.9406
Rice hulls	26		
Premix material	2		
Total	*100*		

[1]The premix provided following per kilogram of diet: VA 8 000 IU; VD3 1 000 IU; VE 50 mg; Lys 1.5 g; Met 1.5 g; Cu 50 mg; Fe 100 mg; Zn 50 mg; Mn 30 mg; Mg 150 mg; Se 0.1 mg.

Biochemical analysis and statistics

Serum samples were analyzed using automatic biochemical analyzer for total protein (TP), alanine amiotransferase (ALT), glucose (GLU), triglyceride (TG), total cholesterol (TCHO), blood urea nitrogen (BUN), immunoglobin G (IgG), immunoglobin A (IgA), immunoglobin M (IgM). These data were collected by Institute for Biochemical Analyses of central hospital in tai'an (Shandong, China).

The counting test of intestine Mast Cells

Intestine sample of rabbits was embbed using conventional paraffin, cutted to 5μm-thick slices by slice cutter and then stained with alcian blue 8GX and 0.018% saffron (Chao, 2005; Morii, 2004). After dying for 30 mins, the slice was washed by distilled water, dehydrated by gradient alcohol, transparented by xylene, sealinged by neutral resin. Observations concerning distribution and morphological features of intestinal mucosal mast cell using light microscopy, five slices were selected for each site to count the number of mast cells.

Statistical analysis

Analyses were performed using AVOVA analysis with SPSS (version 14.0) and differences between means were compared by Duncan's least significant difference at 95% confidence.

Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) experiment

A pair of primer ERIC1 (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') were chosen for ERIC-PCR (Wei, 2004). All the primers used in this study synthesized by Shanghai Biological Engineering Limited Company (Shanghai, China). The ERIC-PCR reaction was carried out in a 50μl volume. DNA was extracted using standard technique from the content of caecum sample at age of 51d and 65d respectively (Cao, 2008). Temperature profile for ERIC-PCR: 95℃ for 7 min; 35 cycles at 94℃ for 1 min, 52℃ for min, 65℃ for 8 min; 65℃ for 16 min, and a final soak at 4℃. Amplified DNA was examined by horizontal electrophoresis in 1.5% agarose gel with 5μl aliquots of PCR products. The dendrogram was constructed using NTSYS-pc 2.10 (Rohlf, 2000) according the result of agarose gel electrophoresis.

RESULTS AND DISCUSSION

The effect of *Lactobacillus* on growth performance

The growth performance of rabbits from weaning to 65d old was showed in Table 2. The data shows that ADFI of experimental group higher than control group respectively, and ADG of group I, II and group III were higher than that of control group from 4.3% , 9.57% to 11.96% respectively. Group I did not differ significantly from control group (P>0.05), however, group II and III differed significantly from control group (P<0. 05). The diarrheal incidence of rabbit lower 16.7%, 13.4% and 6.7% than control group from group I and III. These result dedicated that *Lactobacillus* isolates have a good role on preventing bacteria disease and controlling death rate of weaned rabbit.

Table 2: Effect of *Lactobacillus* isolates on the growth performance and diarrheal incidence of growing rabbits from weaning to 65 d (M±SD, n = 30).

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Growth performance	Control	Group I (antibiotics)	Group II (LB1)	Group III (L3)
ADFI(g)	81.51 ^{a}±0.21	84.23 ^{a}±0.12	85.75 ^{b}±0.17	88.13 ^{b}± 0.13
ADG(g)	20.9 ^{a}±0.15	21.8 ^{b}±0.14	22.9 ^{c}±0.17	23.4 ^{c}± 0.16
F/G	3.90 ^{a}±0.25	3.86 ^{a}±0.16	3.74 ^{b}±0.21	3.77 ^{b}± 0.21
Diarrhea incidence	36.7%	20%	23.3%	30%

*ADG, average daily body weight gain; ADFI, average daily feed intake; F/G, the ratio of feed intake to body weight gain, indicating feeding efficiency. Feed was calculated based on dry weight. ^{a,b} Different superscript letter in the same row were significantly different (P<0.05).

The effect of *Lactobacillus* on serum metabolite

Serum concentrations of TP, ALT, GLU, TG, TCHO, BUN, IgG, IgA, IgM were shown in Table 3. There were no significant differences in serum TP,GLU, TG, TCHO and IgA concentrations among all groups(P>0.05), indicating that *Lactobacillus* had no influence on these parameters. The ALT concentration was decreased significantly (P<0.05) when fed rabbits with *Lactobacillus*. The BUN

concentration decrease significantly when fed rabbits with antibiotics. The concentration of IgG and IgM increased significantly ($P<0.05$). These result dedicated that *Lactobacillus* isolate increase the immunologic function of growing rabbits.

Table 3: Effect of *Lactobacillus* isolates on serum metabolite concentration of growing rabbits of 65d (Mean ± SD, n = 10).

	Control	Group I (antibiotics)	Group II (LB1)	Group III (L3)
TP（g/L）	51.67±9.29	49±6.32	52.5±6.92	52.5±3.39
ALT (IU/L)	51.83 ^{a}±19.03	49.83 ^{a}±18.73	43.66 ^{b}±17.22	39.83 ^{b}±9.92
GLU (mmol/L)	5.31±0.46	5.97±2.61	5.360.97	5.71±0.75
TG (mmol/L)	0.89±0.41	0.82±0.20	0.81±0.34	0.88±0.37
TCHO (mmol/L)	1.03±0.24	1.12±0.25	1.020.47	1.13±0.45
BUN (mmol/L)	7.95 ^{a}±0.66	5.97 ^{b}±0.89	7.82 ^{b}±3.02	7.10 ^{b}±2.54
IgG (IU/L)	1.62 ^{a}±0.06	1.68 ^{b}±0.10	1.74 ^{ab}±0.11	1.71 ^{ab}±0.11
IgA (IU/L)	0.01±0.01	0.01±0.01	0.03±0.01	0.02±0.02
IgM (IU/L)	0.025 ^{a}±0.019	0.025 ^{ab}±0.031	0.042 ^{b}±0.038	0.038 ^{b}±0.020

* TP,total protein; ALT, alanine amiotransferase; GLU, glucose ; TG, triglyceride ; TCHO, total cholesterol; BUN, blood urea nitrogen ; IgG ,immunoglobin G; IgA, immunoglobin A ; IgM, immunoglobin M.
^{a,b} Different superscript letter in the same row were significantly different ($P<0.05$).

The effect of *Lactobacillus* on the number of intestine mast cells

The result about the number of mast cells in different intestine of growing rabbits was listed in Table 4.

Table 4：Effect of *Lactobacillus* isolates on the number of mast cells of growing rabbits at 65d.(Mean ± SD, n = 10).

	Control	Group I (antibiotics)	Group II (LB1)	Group III (L3)
Jejunum（cells/5HP）	31.0 ^{b}±6.1	29.7^{b}±5.1	33.6^{b} +8.8	30.6^{b}±7.8
Duodenum（cells/5HP）	13.6 ^{a}±6.7	14.9 ^{b}±6.8	11.4 ^{a}±5.5	9.7 ^{a}±3.2
Caecum（cells/5HP）	41.2 ^{a}±11.6	39.6 ^{a}±10.9	55.7 ^{b}±13.3	47.8 ^{b}±12.3

^{a,b} Different superscript letter in the same row were significantly different ($P<0.05$).

The result indicated that *Lactobacillus* isolates had no influence on the number of master cells in jejunum and duodenum. The number of master cells in caecum, on the contrast, was increased significantly when fed rabbits with *Lactobacillus* LB1($P<0.05$).This result dedicated that *Lactobacillus* isolate can increase the intestinal immunologic and defensive function of growing rabbits, especially for caecum.

The effect of *Lactobacillus* on intestine diversity of rabbits using ERIC-PCR fingerprinting

The dendrogram showed significant differences among different samples in Figure2. The dendrogram illustrated that feeding rabbits with probiotics isolates and antibiotics all affect the flora and species of intestine. The dendrogram showed the homology of Lane 4 and 8 at 51 days and 65d was 100%, this

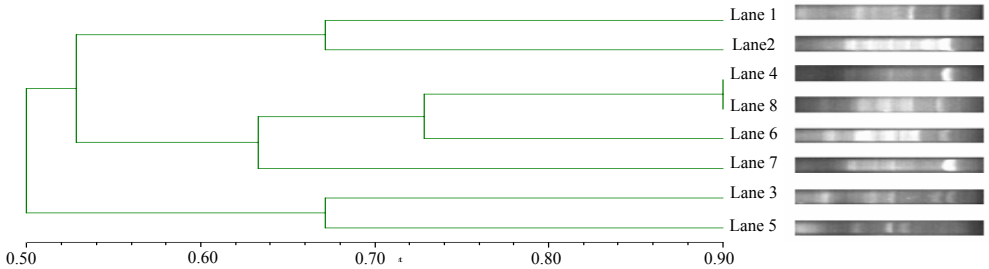

Figure 2：The dendrogram of different groups based on ERIC-PCR at 51d and 65d

[2] Lane1-4:the samples of different group at the age of 51 days; Lane 5-8: the samples of group at the age of 65 days
[3] Lane4.8:ontrol group; Lane 3,7: fed rabbits with *Lactobacillus* LB1; Lane 2,6: fed rabbits with *Lactobacillus* L3; Lane 1,5: fed rabbits with *antibotics*.

result indicated no difference of bacteria distribution was shown at the age of 51d and 65d. Bacteria distribution of other group at the age of 51d and 65d, on the contrast, were different significantly. This result showed that *Lactobacillus* isolates and antibiotics had influence on the number and species of intestinal flora distribution constantly.

CONCLUSIONS

In conclusion, the application of *Lactobacillus* isolates LB1 and L3 all can increase the growth performance, enhance the immunologic function and adjust the intestinal microecosystem of growing Rex Rabbits.

REFERENCE

Cao SY., Wang MS., Cheng AC.,et al. 2008. Comparative analysis of intestinal microbial community diversity between healthy and orally infected ducklings with *Salmonella enteritidis* by ERIC-PCR.. *World J Gastroenterol, 21; 14(7): 1120–1125.*

Chao G., Piao Z., Chen YX.,et al.2005. Regulative Mechanism of Local Immunity in Rat Mammary Gland -Distribution of Mast Cells in Mammary Gland During Lactating and Non-lactating Periods. *Chin J V et S ci ,25(1), 53-55.*

Collado MC., Isolauri E., Salminen S., et al. 2009. The impact of probiotic on gut health. *Curr Drug Metab., 10, 68-78.*

Ley RE., Lozupone C.A., Hamady M., et al.2008. Worlds within worlds: evolution of the vertebrate gut mocrobiota. *Nat Rev Microbiol, 6:776-788.*

Ljungh A., Wadstrom T. 2006. *Lactic acid bacteria* as probiotics. *Curr. Issues Intest. Microbiol. 7, 73-89.*

Li L., Evan F., David C., et al. 2006. Mast cells are essential intermediaries in regulatory T-cell tolerance. *Nature, 442(31), 997-1002.*

Morii E., Ito A., JippoT., Koma Y., et al. 2004. Number of Mast Cells in the Peritoneal Cavity of Mice- Influence of Microphthalmia Transcription Factor through Transcription of Newly Found Mast Cell Adhesion Molecule Spermatogenic Immunoglobulin Superfamily. *American Journal of Pathology, 165(2), 491-499.*

Rohlf, FJ.2000. NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System, version 2.11j. *New York, NY: Exeter Software.*

Wang CY., Wang JQ., Gong J., et al. 2011.Use of *Caenorhabditis elegans* for Preselecting *Lactobacillus* Isolates to Control *Salmonella Typhimurium*. *J.Food Protection.,74 (1),86-93.*

Wei G., Pan L., Du H., et al. 2004. ERIC-PCR fingerprinting based community DNA hybridization to pinpoint genome specific fragments as mo lecular markers to identify and track populations common to healthy human guts. *M icrobio lM ethods, 59(1), 91-108.*