Orally administered *Lactobacillus casei* exhibited several probiotic properties in artificially suckling rabbits

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ABSTRACT

Objective: *Lactobacilli* in rabbit intestine is rare and its function on rabbit gut health is not fully understood. The present study aimed to evaluate *in vivo* the probiotic potential of *Lactobacillus casei* for suckling rabbits.

Methods: Two healthy 5-day-old suckling rabbits with similar weights from each of 12 New Zealand White litters were selected and disturbed to the control group and treatment group. All rabbits were artificially fed. The treatment group had been supplemented with live *Lactobacillus casei* in the milk from the beginning of the trial to 13 days of age. At 15 days of age, healthy paired rabbits were slaughtered to collect intestinal samples.

Results: 1) Oral administration of *Lactobacillus casei* significantly increased the proportion of *Lactobacilli* in the total intestinal bacteria (*P* < 0.01) and obviously reduced that of *Escherichia-Shigella* (*P* < 0.01); 2) treatment increased the length of vermiform appendix (*P* < 0.05); 3) a higher percentage of degranulated paneth cells was observed in the duodenum and jejunum when rabbits administered with *Lactobacillus casei* (*P* < 0.01); and 4) the expression of toll-like receptor 9 (TLR9), Lysozyme (LYZ) and defensin-7-like (DEFEN) in the duodenum and jejunum was stimulated by supplemented *Lactobacillus casei* (*P* < 0.05).

Conclusion: orally administered *Lactobacillus casei* could increase the abundance of intestinal *Lactobacilli,* and exhibited several probiotic properties for suckling rabbit by decreasing the relative abundance of intestinal *Escherichia-Shigella,* promoting the growth of appendix vermiform, stimulating the degranulation of paneth cells and inducing the expression of defensin-7-like and Lysozyme. The results of the present study implied that *Lactobacillus casei* exhibited probiotic potential for suckling rabbit.
Key words: Degranulation, Inflammatory factors, Lysozyme, Paneth cells, Vermiform appendix

INTRODUCTION

Rabbits, especially suckling rabbits and newly weaned rabbits, are susceptible to intestinal infection, which often leads to a serious inflammatory response and death [1-2]. Inclusion of antibiotic in diet is the main strategy to prevent rabbit from pathogenic infection. However, rabbit is herbivore with big cecum, antibiotic supplementation to diet can inhibit cecal fermentation, and thus decrease rabbit’s feed efficiency and growth performance [3]. As the alternative to dietary antibiotics, probiotics have been widely used to prevent intestinal pathogenic infection, and Lactobacilli are the most popular commercial probiotics for different animals. However, Yu and Tsen [4] once reported that the lack of adhesive capability prevented Lactobacilli from colonizing in the intestinal tract of rabbit, and Lactobacilli is rare in any part of rabbit’s gastrointestinal tract [5-6]. Therefore, the use of Lactobacilli in rabbit’s diet is debated. The present study aimed to investigate whether Lactobacilli is an efficient probiotic for rabbits. Generally, probiotic properties for host of a bacterium to the host are evaluated in vitro by testing the probiotic’s antimicrobial potential [7], adhesion ability to the host’s intestinal mucin [8] and resistance to the gastrointestinal environment [9], while the in vivo evaluation of their other probiotic properties, such as the ability to stimulate the development of gastrointestinal immune system development and the ability to regulate intestinal innate immune and inflammation homeostasis, is ignored [10]. Therefore, the probiotic properties of Lactobacilli for suckling rabbit evaluated in vivo in this study.

The vermiform appendix is a unique and important intestinal immunity organ for rabbit [11]. It is well known that the development of intestinal immune system is stimulated by
commensal bacteria. Previous research showed that components from *Bacteroides fragilis* and *Bacillus subtilis* could promote the development of rabbit vermiform appendix, but those from other investigated bacteria, such as *Clostridium subterminale* and *epidermidis*, could not [11]. This suggested that the development of rabbit vermiform appendix is stimulated only by specific commensals, but it is unclear whether *Lactobacilli* belongs to these specific commensals.

Paneth cells are important innate immune cells. Evidence has proven that they play an important role in protecting the small intestine against pathogenic infection and in regulating the intestinal microbial density by releasing antimicrobial α-defensin, lysozyme, peptides, α-defensin—and secretory phospholipase A2 [12]. Regulation of the secretory function in Paneth cells is also involved in the physiological interactions between commensal bacteria and their host. For example, degranulation in mouse Paneth cells has been observed after mice were orally treated with both live bacteria and killed bacteria [13-14]. Stimulatory effect of *Lactobacilli* on the in vitro expression of human β-defensin-2 in Caco-2 cells and on the in vivo expression of porcine β-defensin-2 in piglets was detected [15-16]. The results of these studies suggested that an important property of probiotics is their role in regulating intestinal antimicrobial activity of the host.

The intestinal inflammatory response is also involved in the interaction of commensal bacteria and their host. Our recent study showed that total intestinal bacteria from the rabbit’s intestine tends to induce a higher inflammatory level in cultured crypt and villus of rabbit than that total intestinal bacteria from chickens or pigs [15], probably because of the low abundance of *Lactobacilli* in the rabbit’s intestine—because it was reported that intestinal dsRNA can alleviate intestinal inflammation, and *Lactobacilli* have much higher level of dsRNA than other investigated bacteria [16]. Therefore, the possible function of orally

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administered L. casei in mediating the inflammatory level in rabbit’s intestine is worth to be investigated.

For the above-mentioned reasons, the present study was conducted to evaluate in vivo the probiotic potential of Lactobacillus casei (one of the widely used lactobacillus probiotics) for suckling rabbits by investigating the effects of Lactobacillus casei RABX1, which was previously isolated from the rabbit intestine in our laboratory and selected based on the evaluation in vitro for probiotic properties, including high adhesion ability to rabbit intestinal mucin and strong resistance to the gastric acid and intestinal cholate, and antibacterial ability to Escherichia coli [17]. Its effects on the development of the appendix vermiform, degranulation of paneth cells, expression of defensin-7-like and lysozyme, and the inflammatory response in artificially suckling rabbits were investigated in present study.

MATERIALS AND METHODS

Animal care

All animal protocols were pre-approved by the Animal Protection Committee of Northwest A&F University, and the use of animals and the experimental procedure was consulted to the Guide for the Care and Use of Laboratory Animals of NIH.

Selection of suckling rabbits and feeding experiment

Twelve newborn litters of New Zealand White were selected based on doe’s parity and health. Two healthy male kits with similar body weights were selected from each of these 12 litters at 5-day-old and then randomly distributed into the control group and treatment group. The body weight difference between the two kits in the same pair is < 2.0 g at 5 days of age. All the selected kits were artificially fed with milk from the beginning to the end of the trial. All the kits were fed five times (8:00 am, 12:00 am, 4:00 pm, 8:00 pm and 12:00 pm) every day with a commercial milk powder (without probiotic in the powder) for pet infant. The milk powder was dissolved in warm (37-38°C) boiled water (milk powder: water = 1 g: 4 mL)
before feeding. The volume of the dissolved milk fed to each kit each time was 5 mL (5 d), 8 mL (6 d), 10 mL (7-8 d), 15 mL (9-11 d), 20 mL (12-13 d) and 25 mL (14-15 d). Kits in the treatment group had been continuously supplemented with *Lactobacillus casei* RABX1 (accession number: KT944253) from 5 days of age to 13 days of age. To prevent the interference from the supplemented *Lactobacilli*, which would affect the investigated percentage of intestinal *Lactobacilli*, which would affect the investigated percentage of intestinal *Lactobacilli*, the isolated bacterium was cultured in sterile Mann-Rogosa-Sharp broth at 37°C for 12 h and harvested by centrifugation at 5000g for 6 min and then washed with sterile phosphate buffered saline. The number of the harvested bacteria was estimated by a spectrophotometer. Bacteria were then resuspended in the milk to reach a concentration of 5-6 × 10⁸ Colony-Forming Units (CFU)/mL and orally administered to kits in the treatment group. All the kits were caged in boxes with constant temperature of 30°C (moisture 50-70%). The kits of the same group were caged in the same box with natural lighting. The padding towels were changed twice every day and sterilized in boiled water after being washed, and the boxes were cleaned three times every day (before feeding). All the kits were weighed at 5 days and 15 days of age before morning feeding, and their health condition was recorded every day, the unhealthy kits were separated from its group as soon as their abnormality were found.

**Sampling and measurement of vermiform appendix length**

Healthy pairs of kits were slaughtered eight hours after the morning feeding at 15 days of age. Before sampling, the length of the vermiform appendix was quickly measured from its end to the junction between the cecum and vermiform appendix. Then, the content in small intestine was collected and mixed together to determine the relative proportion of intestinal *Lactobacilli* or *Escherichia-Shigella* in total bacteria (Note: the content in kit’s duodenum, jejunum and ileum wasn’t collected separately because it was too less). Two parts
each were harvested from the duodenum, jejunum and ileum. One part was stored at -80°C for
determination of gene expression, and the another part was fixed in 4% paraformaldehyde for
investigation of the intestinal morphology and degranulation of paneth cell.

**Determination of the relative proportion of ileal Lactobacilli and Escherichia-Shigella in total bacteria**

Total bacterial genomic DNA in the collected intestinal content was extracted by the
modified phenol-chloroform-isoamylalcohol extraction method [1218]. The concentration of
the extracted DNA solution was subsequently determined and then diluted to 15 ng/μL. The
relative proportion of Lactobacilli or Escherichia-Shigella in total bacteria was determined by
relative quantification real-time polymerase chain reaction (PCR). The reaction mixture (20
μL) consisted of 10 μL of SYBR Premix Ex Taq (Takara Biotechnology (Dalian) Co., Ltd,
Dalian, Liaoning, China), 0.4 μM of each primer and 30 ng of the extracted bacterial genomic
DNA. The average cycle threshold (Ct) value for Lactobacilli, Escherichia-Shigella or total
bacteria in each sample was determined in triplicate. Then, the average Ct value of
Lactobacilli (or Escherichia-Shigella) after being normalized to that of total bacteria was used
for calculating the relative proportion of Lactobacilli (or Escherichia-Shigella) in total
bacteria by using the 2-ΔΔCt method as previously described [1218]. Real-time PCR was
performed on the IQ5 Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The primers
used for detecting 16S rDNA of Escherichia-Shigella, Lactobacilli and total bacteria are
listed in Table 1.

**Measurement of intestine morphology and quantitation of paneth cell degranulation**

The fixed intestine sections were rinsed with water and then dehydrated in a graded series
of ethanol. After being cleared in benzene twice, sections were saturated with paraffin first
and then embedded in paraffin. The embedded sections were cut into thin slices (5 μm
thickness, 10 slices of each sample), which were stained with hematoxylin/eosin and prepared
for observation by light microscopy. A total of 10 intact, well-oriented crypt-villus units were
selected in triplicate for each intestinal cross-section. Villus height was measured from the tip
of the villus to the villus-crypt junction. Crypt depth was defined as the depth of the
invagination between two adjacent villi. The intestine morphological measurements were
performed by using an image processing and analysis system (Optimus software version 6.5,
Media Cybernetics, North Reading, MA).

Quantitation of degranulated Paneth cells was performed following the method described
by Rumio et al. [14](2012). The fixed intestinal sections were embedded in paraffin and then
cut into 5-μm slices. After staining with hematoxylin and eosin (H&E), the slices were
mounted in Entellan® (Merck, Darmstadt, Germany) and then observed with a ViCo
microscope (Biomedica Mangoni S.n.C., Nikon Instruments S.p.A, Pisa, Italy) equipped with
a digital Nikon DS-L1 camera. Paneth cells were quantitated only in crypts that were
sectioned through their center and could present a clear lumen. Degranulated Paneth cells
were distinguished from non-degranulated Paneth cells based on the increased granule
dimension and presence of large darkly colored vacuoles in the cytoplasm [14](Rumio et al.,
2012). The percentage of degranulated Paneth cells in each crypt was calculated, and twenty
crypts were analyzed for each intestinal segment.

**Determination of gene expression by quantitative RT-PCR**

Total RNA of each intestine sample stored at -80°C was extracted using Trizol reagent
(Invitrogen, Carlsbad, Ca, USA). The cDNA was synthesized from 1 μg of total RNA using a
reverse transcriptase kit (Takara Biotechnology (Dalian) Co., Ltd, Dalian, Liaoning, China).
The relative mRNA levels of TLR9, DEFEN, LYZ, tumor necrosis factor alpha (TNF-α),
interferon beta (IFN-β) and interleukin 6 (IL-6) in the intestine were quantified by real-time
quantitative PCR, which was carried out on the IQ5 Cycler using the TaKaRa SYBR Premix
Ex Taq kit. The reaction system contained 1 μL of synthesized cDNA, 1 μL of each primer (4
μM), 10 μL of SYBR Premix Ex Taq (Takara Biotechnology (Dalian) Co., Ltd, Dalian, Liaoning, China) and 7 μL of nuclease-free water. The cycle threshold (Ct) value for the investigated gene in each sample was determined in triplicate, and the average Ct value was calculated. Finally, the average Ct values for each gene, after being normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, were used for quantification by the 2−ΔΔCt method. Real-time PCR was carried out with an initial denaturation step of 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 45 s. The primers designed using Primer 5.0 or referenced are shown in Table 2.

Statistics

The data collected from the experiment were analyzed with a paired-samples t test using SPSS19.0 soft package (SPSS Inc, Chicago, IL, USA), and the results were presented as mean ± standard deviation (SD) for each trait. The probability of significance is indicated by the following conventional standard abbreviations: $P > 0.05$ for non-significance and $P < 0.05$, $P < 0.01$ and $P < 0.001$ for significance at these levels.

RESULTS

During the feeding experiment, two kits in control group and three in treatment group died at different time, all the dead kits came from the pairs with lighter initial body weight, and they died of diarrhea (one in control, one in treatment) or no symptoms (one in control, two in treatment). One dead kit in control group and one dead kit in treatment group came from the same litter. One dead kit in treatment group, therefore, all the following investigated data were collected from eight healthy kit pairs of kits. There was no difference in average daily gain (ADG) between the two groups ($P = 0.759$) (data not shown).

Effect of treatment on the proportion of Lactobacilli or Escherichia-Shigella in total
The relative proportion of the investigated bacterium in total bacteria was measured by real-time PCR. As presented in Table 3, although the oral administration of *Lactobacillus casei* had been stopped for more than 24 h, suckling rabbits in the treatment group had an extremely higher relative proportion of *Lactobacilli* in intestinal bacteria (*P* < 0.001) and an extremely lower relative proportion of *Escherichia-Shigella* (*P* < 0.001) than rabbits in the control group.

**Effect of treatment on intestinal morphology, vermiform appendix length and percentage of degranulated paneth cells**

Oral administration with *Lactobacillus casei* did not change the intestinal morphological indices, including villus height, crypt depth and the ratio of villus height to crypt depth (*P* > 0.05), but it significantly increased the length of the vermiform appendix in suckling rabbits (*P* = 0.04) (Table 4). Degranulated paneth cell was showed in Supplementary Figure S1. As presented in Figure 1, the percentage of degranulated paneth cells in the duodenum and jejunum of suckling rabbits orally administered with *Lactobacillus casei* RABX1 was increased by 206% and 177% (*P* < 0.001), respectively. Degranulation of paneth cells was also observed in ileum sections, but the difference in the percentage of degranulated paneth cells between the two groups was not obvious (19 ± 6.1% in the control group vs. 25 ± 5.7% in the treatment group).

**Effect of oral administration with *Lactobacillus casei* on expression level of the investigated genes**
Expression of TLR9, defensin-7-like and LYZ in the duodenum (P < 0.001, P = 0.05 and P = 0.008, respectively) and jejunum (P < 0.001, P < 0.001 and P = 0.001, respectively) was significantly increased by orally administered Lactobacillus casei RABX1 (Figure 2), but that in the ileum was not affected (P > 0.05). Oral administration with Lactobacillus casei did not change the expression levels of TNF-α, IFN-β and IL-6 in the duodenum, jejunum and ileum of artificially suckling rabbits (Figure 3).

DISCUSSION

Probiotic properties have bacterium-host specificity. Unlike in the intestines of many animals such as chicken, the dominant genus in the intestine of rabbit is not Lactobacilli, which occupies less than 1% of the total intestinal bacteria in rabbit [19]. It is necessary to confirm whether Lactobacilli can be used as an effective probiotic for rabbits. Therefore, the present study explored in vivo the probiotic properties of Lactobacillus casei in suckling rabbit. The development of intestinal microflora begins at the day of birth and is affected by many factors [22-23], including the bacterial structures in the mother’s birth canal or milk, so we designed a paired experiment and artificially fed suckling rabbits from the fifth day after their birth to minimize the influence from their mother. Kits were not separated from their mother before 5 days old because we found that kits could not survive if they did not take up suck the their mother’s colostrum.

Considering the strain specificity effect of probiotics, we evaluated in vitro the adhesion ability to rabbit intestinal mucin and resistance to the gastrointestinal environment of several Lactobacilli species, and the strain Lactobacillus casei RABX1 exhibited good properties for the in vitro evaluated indices. To make sure that the investigated Lactobacilli was not the administered Lactobacillus casei RABX1 themselves, administration of Lactobacillus casei RABX1 in treatment group was stopped after 13 days of age. The fact that the relative
proportion of Lactobacilli in total bacteria (detected after the administration of Lactobacillus casei RABX1 had been stopped for about 40 h) in the treatment group extremely higher than that in the control group indicated that Lactobacillus casei RABX1 could effectively get to the small intestine of artificially suckling rabbits and be proliferous there. Probiotics can suppress pathogens through different mechanisms [2324], including competition with pathogens for nutrients and inhibition of pathogens by producing antimicrobial metabolites. The decrease in Escherichia-Shigella in the intestine of suckling rabbit should be related to the suppression suppressive function of increased intestinal Lactobacilli.

The development of the vermiform appendix is associated with the immune capacity of the intestine of a rabbit’s intestine. Štěpánková et al. [2425] reported that normal rabbits had a much better developed vermiform appendix than germ-free rabbits, and our recent study found that suckling rabbits with more opportunity to have contact with their mother had more intestinal commensal bacteria and longer vermiform appendices [2526]. These two research studies indicated that the development of the special intestinal immune organ in rabbits needs bacterial stimulation. Rhee et al. [11] proved that it was special commensals such as Bacteroides fragilis and Bacillus subtilis that could be involved in stimulating the development of the vermiform appendix. Our study showed that orally administered Lactobacillus casei also promoted the development of the vermiform appendix in suckling rabbit.

Degranulation is the way that a paneth cell secretes its synthesized antimicrobial substances, such as defensin and lysozyme, to the intestinal lumen [14]. The action of degranulation is regulated by the TLR9 signaling pathway, which can be stimulated by a special fragment of bacterial DNA [2627]. The increased percentage of degranulated paneth cells and expression levels of TLR9, defensin-7-like and lysozyme in the duodenum and jejenum in the present study indicated that exogenous Lactobacillus casei was involved in the
regulation of paneth cell function in suckling rabbits and suggested another probiotic property of *Lactobacillus casei* for rabbits. The expression of TLR9, defensin-7-like and lysozyme in the ileum was not affected by treatment, which was inconsistent with the duodenum and jejunum. This inconsistency was probably derived from the uneven distribution of paneth cells in different intestinal segments. It was reported that most paneth cells locate in the forepart of the small intestine [12], and our morphological test also showed that paneth cells were rare in the rabbit ileum.

Components of commensal bacteria can alleviate intestinal inflammation by regulating the expression of prepro-inflammatory factors and anti-inflammatory factors. Kawashima et al. [27] reported that bacterial double-stranded RNA (dsRNA) showed a regulatory function by triggering anti-inflammatory factor IFN-β production and inhibiting prepro-inflammatory factor production, and they also proved that *Lactobacilli* contains higher dsRNA than other investigated bacteria. Furthermore, it was reported that TLR9 signaling, which was stimulated by orally administered *Lactobacillus casei* in the intestine of suckling rabbit [28], had an anti-inflammatory effect on murine experimental colitis [28]. However, increased intestinal *Lactobacilli* here neither induced higher expression of the anti-inflammatory factor IFN-β nor inhibited that of prepro-inflammatory factors. It meant that the combined effect of the supplemented *Lactobacillus casei* did not alter inflammation homeostasis in the intestine of healthy suckling rabbits. Subsequent research should investigate the probiotic effect of *Lactobacillus casei* supplementation on the anti-inflammatory response in suckling or newly weaned rabbits when they are suffering from intestinal inflammation.

In conclusion, orally administrated *Lactobacillus casei* decreased the relative proportion of *Escherichia-Shigella* in total intestinal bacteria, increased the relative proportion of *Lactobacilli* in total intestinal bacteria, stimulated development of the appendix.
vermiform, and induced degranulation of Paneth cells and the expression of TLR9 and lysozyme in suckling rabbit. In conclusion, although Lactobacilli is not abundant in the rabbit’s intestine, orally administered Lactobacillus casei could effectively increase the abundance of intestinal Lactobacilli and exhibit several probiotic properties for suckling rabbit. However, it didn’t improve the growth performance during the experimental period. Further study should be conducted to investigate the subsequent positive effect of early administrated Lactobacillus casei on newly weaned and/or growing rabbit.

CONFLICTS OF INTEREST

All authors approved the submission of this manuscript and had no competing interests to declare regarding this work.

ACKNOWLEDGEMENTS

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REFERENCES


Table 1 The specific primers for bacteria

<table>
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<tr>
<th>Items</th>
<th>Primer</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
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<td></td>
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<td>96</td>
<td>[20,21]</td>
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Notes: GAPDH, TLR9, LYZ, DEFEN, TNF-α, IFN-β and IL-6 are abbreviations for glyceraldehyde-3-phosphate dehydrogenase, toll-like receptor 9, Lysozyme, defensin-7-like, tumor necrosis factor alpha, interferon beta and interleukin 6, respectively.
Table 3 Effect of treatment on the relative proportion of *lactobacillus* and *Escherichia-Shigella* in total bacteria

<table>
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<th>Item</th>
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<th>Treatment</th>
<th>P-value</th>
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<tbody>
<tr>
<td><em>Lactobacillus</em></td>
<td>0.90 ± 0.14</td>
<td>116.14 ± 19.46</td>
<td>&lt; 0.001</td>
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<tr>
<td><em>Escherichia-Shigella</em></td>
<td>30.37 ± 4.60</td>
<td>1.20 ± 0.22</td>
<td>&lt; 0.001</td>
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Table 4 Effects of *Lactobacillus casei* on intestinal morphology and appendix length of suckling rabbits.

<table>
<thead>
<tr>
<th>Item</th>
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<tr>
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<td>384.49±25.91</td>
<td>365.19±28.41</td>
<td>0.668</td>
</tr>
<tr>
<td>Jejunum</td>
<td>386.23±16.03</td>
<td>396.17±17.75</td>
<td>0.703</td>
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<tr>
<td>Ileum</td>
<td>369.73±17.99</td>
<td>337.61±31.34</td>
<td>0.427</td>
</tr>
<tr>
<td>Crypt depth (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>68.10±4.16</td>
<td>67.82±2.41</td>
<td>0.962</td>
</tr>
<tr>
<td>Jejunum</td>
<td>63.32±2.87</td>
<td>63.36±2.50</td>
<td>0.990</td>
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<tr>
<td>Ileum</td>
<td>50.73±2.51</td>
<td>50.38±3.71</td>
<td>0.924</td>
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<tr>
<td>V/C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>5.71±0.34</td>
<td>5.40±0.41</td>
<td>0.612</td>
</tr>
<tr>
<td>Jejunum</td>
<td>6.22±0.44</td>
<td>6.28±0.28</td>
<td>0.903</td>
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<tr>
<td>Ileum</td>
<td>7.37±0.43</td>
<td>6.86±0.70</td>
<td>0.528</td>
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</table>

**Vermiform** Appendix length (cm)

<table>
<thead>
<tr>
<th>Item</th>
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<th>Treatment</th>
<th>P-value</th>
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<td>V/C</td>
<td>4.22±0.18</td>
<td>4.96±0.33</td>
<td>0.044</td>
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</tbody>
</table>

Note: V/C is the abbreviation for villus height/crypt depth.
**Figure 1** Effect of orally administered *Lactobacillus casei* (treated) on the percentage of degranulated paneth cells in the intestine of suckling rabbits.

**Figure 2** Effect of orally administered *Lactobacillus casei* (treated) on the relative expression of TLR9 and LYZ in the intestine of suckling rabbits.  
Note: TLR9 and LYZ are abbreviations for Toll-like receptor 9 and lysozyme, respectively.

**Figure 3** Effect of orally administered *Lactobacillus casei* (treated) on the relative expression of TNF-α, IFN-β and IL-6 in the intestine of suckling rabbits.  
Note: TNF-α, IFN-β and IL-6 are abbreviations for tumor necrosis factor alpha, interferon beta and interleukin 6, respectively.
figure 1
figure 2
Figure 3