Effect of *Lactobacillus Rhamnosus* GG on the Number and Differentiation of T Lymphocytes in the Intestine of Early Weaned Piglets

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**Abstract**

**Background:** Probiotics are defined as living microorganisms by the United Nations (FAO) and the World Health Organization (WHO). When appropriate quantities are used, probiotics will have beneficial effects on human and animal bodies. Probiotics can be used to prevent and treat intestinal infection, urogenital diseases, bacterial vaginosis and skin diseases.

**Methods:** In this study, *Lactobacillus rhamnosus* GG (LGG) ATCC53103 was selected as the experimental strain. Sixty-five-day healthy piglets were randomly divided into two groups, the experimental group LGG group and the control group PBS group. The piglets in LGG group were given 1 ml LGG (6 × 10⁹ cfu/ml) every day. The piglets in the PBS group were given 1 ml PBS every day. Piglets were slaughtered on the 15th day, and the mesenteric lymph nodes (MLN), ileum and Peyer’s patches (PPs), ileum and jejunal lamina propria (LP) were taken. The expression of CD3⁺CD4⁺ T lymphocytes and CD4⁺CD8⁺ double positive T lymphocytes were detected by flow cytometry and immunofluorescence.

**Results:** The results of flow cytometry showed that there was no significant difference in CD3⁺CD4⁺ T lymphocytes in the MLN of the LGG group compared with the PBS group (P > 0.05). The CD3⁺CD4⁺ T lymphocytes in the jejunum and ileum of the LGG group were significantly different from those in the PBS group (P < 0.05). In the LGG group, CD3⁺CD4⁺ T lymphocytes in the ileal LP of piglets were significantly different from those in the PBS group (P < 0.01). The CD4⁺CD8⁺ T lymphocytes in the IEL of the LGG group were significantly higher than those in the PBS group (P < 0.001).

**Conclusion:** In summary, LGG can stimulate the increase in the number of T lymphocytes in piglets and tend to differentiate into Th1.

**Keywords**

Piglet immune function, *Lactobacillus rhamnosus*, P40 protein, T lymphocytes

**Abbreviation**

BSA: Bovine Serum Albumin; DMSO: Dimethyl Sulfoxide; EDTA: Ethylene Diamine Tetraacetic Acid; PMA: Phorbol Myristate Acetate; FCM: Flow Cytometry; LGG: *Lactobacillus rhamnosus* GG; MLN: Mesenteric Lymph Nodes; PPs: Pyle’s Knot; LP: Lamina Propria; Treg: Regulatory Cell; IEC: Small Intestinal Epithelial Cell; Th: T Helper Cells; TCR: T Cell Receptor; IL: Interleukin; IFN: Cervical Lymphnodes; TGF: Transforming Growth Factor

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Introduction

In recent years, with the rapid development of China’s breeding industry, the weaning age of piglets is also in advance [1]. The weaning time of piglets determines the number of piglets per year and the reproduction rate of sows [2]. At the same time, the earlier weaning the piglets, the lower the production cost. However, early weaning of piglets will have a series of adverse effects on piglets. First of all, piglets will suffer from malnutrition due to early weaning, and their growth and development will be affected. Secondly, breast milk contains a large number of antibodies. Early weaning will cause low immunity, intestinal damage, diarrhea and so on. It is reported that the economic loss caused by early weaning of piglets in China is nearly 6 billion yuan every year, which has a great impact on the development of China’s breeding industry. So it is an urgent problem to improve the immune function of early weaned piglets. T cells have specificity in immunity. T cells mediate inflammation by releasing cytokines, so as to improve immunity.

As a model probiotic, LGG has become the first choice in probiotic experiment. LGG is a probiotic isolated from feces of healthy adults. It has the characteristics of most probiotics. A large number of experiments show that LGG has high antibacterial activity and high resistance to gastric acid [3]. At present, there are few data about the effect of LGG on piglet health. LGG can replace and inhibit pathogens, including Salmonella, Clostridium and Escherichia coli. This shows that LGG is one of the candidate strains to improve the immunity of piglets [4].

Probiotics are nonpathogenic microorganisms of gastrointestinal flora. They change the intestinal microbiota to give health to the body. Generally, bifidobacteria and other members of resident microbiota are selected as probiotics. Lactobacillus rhamnosus GG (LGG) is a kind of safe probiotics, which is relatively resistant to acid and bile. It can adhere to epithelial cells and intestinal mucus in vitro, form antibacterial substances and instantly colonize human intestine [5]. The mechanism is that LGG can regulate intestinal flora [6]. Some experiments showed that healthy piglets were infected with E. Some experiments also found that LGG has the effect of eradicating the maladjustment of infant pathogenic flora [7]. A large number of experiments have proved that LGG can regulate intestinal flora, which makes LGG become one of the most popular probiotics.

Intestinal microflora is a very complex group. The number and species of colonized bacteria are different in different parts of human intestine. There are about 1014 bacteria in the intestine of a healthy adult, which are composed of 30 genera and 500 species. The organism has evolved over thousands of years and these bacteria have evolved along with the organism. There has been a symbiotic relationship between organism and flora. Flora is now considered to be the most distinctive function in the body, that is to regulate gene expression, participate in mucosal barrier defense, and also regulate intestinal development and maturity [8]. Intestinal flora is not only closely related to intestinal diseases, but also causes systemic diseases. It has been proved that the intestinal flora of the mouse model of rheumatoid arthritis is in a state of disorder [9], and the imbalance of intestinal flora has an inseparable relationship with diabetes [10]. Diarrhea of piglets caused by dysbacteriosis caused by early weaning has seriously harmed the pig industry in China. At present, human fecal bacteria transplantation technology is gradually accepted and recognized by people, which shows that intestinal flora has been paid more and more attention. Innate immunity is also called nonspecific immunity. As the first barrier of human body, innate immunity is of great significance to the body [11]. Specific immunity is established on the basis of natural immunity. Enhancing innate immunity is an important aspect of improving the whole immunity of the body. LGG regulates innate immune response by regulating phagocyte. Macrophages have Toll like receptors and nucleotide binding oligomerization domain like receptors, so they can detect invading bacteria and viruses. They respond by producing cytokines, the most important of which are IL-1, IL-12, IL-23 and TNF-α. LGG can prevent or improve inflammation of chronic colitis [12]. Because the excessive production of pro-inflammatory cytokines, especially TNF-α, is related to the pathogenesis of chronic intestinal inflammation, [13] LGG was found to enhance macrophage adhesion.

Material and Methods

Strain

Lactobacillus rhamnosus atcc53103 was preserved by the school of animal science and technology of Jilin Agricultural University (Jilin Province animal microbiological preparation engineering research center) and purchased in the United States model culture repository.

Experimental animals

Six 25-day-old healthy piglets of junmu No.1 were purchased from the breeding pig farm of Jilin University. The piglets were randomly divided into two groups, three in each group, and fed with sterile drinking water and antibiotic free feed.

Main reagents

Dithiothreitol (DTT), sodium chloride, 300 mesh screen, 600 mesh screen and anhydrous ethanol were purchased from Beijing chemical preparation company. Tween 80, neutral protease, mouse collagenase IV type I DNease I, and red blood cell lysate were purchased from biyuntian company. Sodium azide, Percoll cell separation solution, PHA, PMA and ionomycin were purchased from
syringe until it is fully ground, then suck the liquid into 1.5 ml EP tube, put it into the precooled centrifuge, at 4 °C, 2000 rpm, and centrifuge for 5 min. Then discard the supernatant to obtain lymphocytes. After washing twice with FACS buffer, the cells were resuspended with 1 ml pbs and counted by cell counting plate after dilution.

**Preparation of LP and IEL single lymphocyte suspension**

Take the jejunum and ileum for 10 cm, remove the excess fat, place them in PBS -/- precooled at 4 °C, cut the intestine longitudinally, and clean the intestine with PBS -/- precooled until it is cleaned. Then, the intestine was cut into about 1 cm with ophthalmic scissors. Put the cut intestine tube into 5 ml of IEL separation solution, vibrate at 37 °C for 15 min at constant temperature (200R/min), and put the intestine tube on 200 mesh nylon filter screen to discard the waste liquid. Repeat. The filtrate was single cell suspension of IEL, which was collected, counted and stained. Put the remaining intestinal tube into 5 ml of the inherent layer of lymphocyte separation solution, vibrate at 37 °C for 45 min with constant temperature (200 R/min); filter the intestinal tube into a 300 mesh sterile filter screen, discard the so-lid residue, put the filtrate into a 15 ml sterile centrifuge tube, centrifugate at 4 °C, 400 × g, for 10 min, discard the supernatant and collect LP cells. Take a new 15 ml centrifuge tube, place 4 ml 80% isotonic Percoll solution at the bottom of the 15 ml centrifuge tube, and then resuspend LP cells with 7 ml 40% isotonic Percoll solution. The LP cells were drawn with a 1 ml syringe, spread on 80% isotonic Percoll solution slowly and evenly, and centrifuged at 2300 rpm for 20 min at room temperature. Suck out the cell layer with two layers of liquid surface, transfer it into a new 15 ml centrifuge tube, add PBS -/- to wash twice, and count the cell count plate after dilution.

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**Main instruments**

<table>
<thead>
<tr>
<th>Table 1: Instruments and Equipments.</th>
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<td><strong>Equipment name</strong></td>
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<tr>
<td>SW-CJ-2FD Single-sided double clean bench</td>
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<tr>
<td>ME204 Electronic analytical balance</td>
</tr>
<tr>
<td>CJ78-1 Type magnetic heating stirrer</td>
</tr>
<tr>
<td>HRLM-80 Full-automatic autoclave</td>
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<tr>
<td>BCD-649WDCEType refrigerator</td>
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<tr>
<td>G70D20CN1P-D2(S0) 20L Microwave oven</td>
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<tr>
<td>HERA cell 240/37 °C CO₂ constant temperature incubator</td>
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<tr>
<td>Innova 40RT Type constant temperature shaker</td>
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<td>5804R Desktop large capacity refrigerated centrifuge</td>
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<tr>
<td>Flow cytometry (LSR-FORTESA)</td>
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<td>Real-time PCR instrument</td>
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**Main instruments**

**Method**

**Preparation of bacterial solution**

The stored LGG atcc53103 was opened in the super clean platform and inoculated on MRS medium. Put it into the 37 °C incubator of the anaerobic tank for 12 h, pass it for three generations continuously, dilute it in the growth logarithmic period, count the colonies, and finally convert the colony forming units (CFU/ml).

**Grouping of experimental animals**

Six healthy piglets were randomly divided into two groups, three in each group. One group was PBS group, the other group was LGG intervention group. The PBS group was given 1 ml PBS for 15 days. LGG group was administrated with 1 ml gg (10⁹ cfu/ml) for 15 days. At the 15th day, the piglets were slaughtered. The mln, jeju-nal PPS, ileal PPS, ileal lamina propria (LP) and jejunal lamina propria (LP) of the piglets were taken aseptically, and the single cell suspension was prepared. The num-ber of T-lymphocytes was detected by flow cytometry, and the number of T-lymphocytes was detected by immuno-fluorescence method.

**MLN, PPS single cell suspension preparation method**

In the ultra clean platform, MLN and PPS were stripped with ophthalmic scissors and ophthalmic forceps (Autoclaved), and the excess fat was removed. Put the folded 200 mesh sterile filter screen into a sterile plate, and add 1 ml rpmi-1640 culture medium. Put the tissue into the filter screen, gently grind the end of sterile 1 ml syringe until it is fully ground, then suck the liquid into 1.5 ml EP tube, put it into the precooled centrifuge, at 4 °C, 2000 rpm, and centrifuge for 5 min. Then discard the supernatant to obtain lymphocytes. After washing twice with FACS buffer, the cells were resuspended with 1 ml pbs and counted by cell counting plate after dilution.

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**Antibody staining**

Take 100 µL (1 × 106 cells) of the above cells, and put them into 1.5 ml centrifuge tube. At the same time,
a blank control tube and a single positive tube were set up; 10 µl of diluted antibodies were added to the corresponding cells, fully mixed, and incubated at 4 °C for 30 min in dark; 1 ml PBS was added to wash twice, and the cells were resuspended with 300 µl facs solution; the cells were put into the flow tube through 300 mesh nylon membrane and detected on the computer.

Immunofluorescence

The ileum and PPS fixed in 10% formaldehyde solution were rinsed with running water for 24 hours, and then the tissue gradient was dehydrated and transparent. Then the tissue was soaked in wax and embedded in paraffin. The tissue was cut into 6 µm with a microtome, placed on a slide, and dried in a constant temperature oven at 80 °C. The sections were dewaxed and hydrated, then the antigen was repaired by the repair solution, and then soaked in PBS for 5 minutes. Add the breaker to break the membrane, then seal it with BSA for 1 h, circle the tissue with an immune histochemical pen, add the antibodies, incubate it at 4 °C in dark for 30 min, and wash it with PBS for 3 times. Then add diluted DAPI, dye for 3 min, wash with PBS for 3 times. Sealing agent and anti-fluorescence quenching agent are added to the tissue and sealed with cover glass. Observe under fluorescence microscope.

Lymphocyte proliferation

Take the above MLN cells (1 × 105 cells) and spread them in 96 well plate, add PHA in each empty space, put them in cell incubator for 44 h, take out the cells, add MTs in each hole, and then put them in cell incubator for 4H. Detect the OD value.

RNA extracted from CD3+ CD4+ T lymphocytes

By flow cytometry, CD3+ CD4+ T lymphocytes in each group were sorted out. According to the instructions of cell RNA extraction kit, RNA of CD3+ CD4+ T lymphocytes was extracted. The concentration was adjusted to be the same, reverse transcribed into cDNA, and stored at - 80 °C.

Fluorescent quantitative PCR

The reverse cDNA was placed in octet respectively, and the reaction system was prepared according to the instructions of fluorescent quantitative PCR kit. Put it on the fluorescence quantitative PCR instrument for reaction, and collect the data when the reaction is over.

Bioinformatics and statistical analysis

The data were analyzed by graph pad prism 5.0 software. Compared with the data of the two groups, the student’s t test was used; compared with the data of the two groups, the multiple comparison method in one-way ANOVA was used for analysis, and the difference was statistically significant (P < 0.05).

Results

The effect of LGG on T-lymphocyte differentiation in piglets

As a model probiotic, LGG can regulate the immune system, treat and prevent diarrhea. Therefore, LGG was selected as the test strain in this experiment, and 25 day old piglets were given gavage for 15 days. After slaughter, the intestinal tract and lymph nodes were taken out aseptically. The number of T lymphocytes was detected by flow cytometry, and CD3+ CD4+ T lymphocytes were selected. The transcription factors of CD3+ CD4+ T lymphocytes were detected by fluorescence quantitative PCR. To further study the effect of LGG on the immune function of piglets.

Effect of LGG on lymphocyte proliferation in piglets

The MLN of LGG15d piglets was taken and made into a single cell suspension. The lymphocyte proliferation in MLN was detected by MTS method, and the results are shown in Figure 1. The lymphocyte proliferation in MLN of piglets fed with LGG ATCC53103 was significantly higher than that of the normal PBS group (P < 0.001).

The results showed that LGG could stimulate lymphocyte proliferation in piglets.

Effect of LGG on the number of CD3+ CD4+ T lymphocytes in MLN of piglets

The MLN single cell suspension of piglets was stained with antibody, and the number of CD3+ CD4+ T lymphocytes in MLN of piglets was detected by flow cytometry. From Figure 2, it can be seen that there was no significant difference between the CD3+ CD4+ T lymphocytes in MLN of piglets fed with LGG and the PBS group (P > 0.05). We analyzed that the MLN of newborn piglets was not mature, so we will carry out the next experiment to test Card.
LP of piglets was detected by flow cytometry. From Figure 5, we can see that the number of CD8+ CD4+ T lymphocytes in jejunal PPS of piglets fed with LGGA-TCC53103 was significantly higher than that in PBS group (P < 0.05).

The effect of LGG on the number of CD4+CD8+ T lymphocytes in ileum LP of piglets

We stained the single cell suspension of PPS in the ileum of piglets with antibody, and detected the number of CD8+ CD4+ T lymphocytes in the ileum LP of piglets by flow cytometry. From Figure 6, we can see that the number of CD8+ CD4+ T lymphocytes in the ileum PPS of piglets fed with LGGA-TCC53103 was significantly higher than that in the PBS group (P < 0.01).

The effect of LGG on the number of CD4+ CD8+ double positive T lymphocytes in piglet IEL

The single cell suspension of piglet IEL was stained
Figure 4: The number of CD4\(^+\) CD3\(^+\) T lymphocytes in ileal PPs of LGG piglets at 15d.

CD4\(^+\) CD3\(^+\) T lymphocytes in ileal PPs of LGG piglets at 15d.

Figure 5: The number of CD4\(^+\) CD8\(^+\) T lymphocytes in jejunum LP of piglets fed LGG at 15d.

CD4\(^+\) CD8\(^+\) T lymphocytes in jejunum LP of piglets fed LGG at 15d.

Figure 6: The number of CD4\(^+\) CD8\(^+\) T lymphocytes in the ileal LP of piglets fed LGG at 15d.

CD4 + CD8 + double positive T lymphocytes

CD4 + CD8 + double positive T lymphocytes

CD4 + CD3 + T lymphocytes in ileal

CD4 + CD3 + T lymphocytes in ileal

Figure 4: The number of CD4\(^+\) CD3\(^+\) T lymphocytes in ileal PPs of LGG piglets at 15d.

CD4\(^+\) CD3\(^+\) T lymphocytes in ileal PPs of LGG piglets at 15d.

Figure 5: The number of CD4\(^+\) CD8\(^+\) T lymphocytes in jejunum LP of piglets fed LGG at 15d.

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Figure 6: The number of CD4\(^+\) CD8\(^+\) T lymphocytes in the ileal LP of piglets fed LGG at 15d.

CD4 + CD8 + double positive T lymphocytes

CD4 + CD8 + double positive T lymphocytes

CD4 + CD3 + T lymphocytes in ileal

CD4 + CD3 + T lymphocytes in ileal
with antibody, and the number of CD4\(^+\) CD8\(^+\) double positive T lymphocytes in IEL was detected by flow cytometry, and the situation of the gate was shown in Figure 7. Results as shown in Figure 8, the number of CD3\(^+\) CD4\(^+\) T lymphocytes in PPS of ileum of piglets fed with LGG ATCC53103 (15d) was significantly higher than that of PBS group (P < 0.001).

**Effect of LGG on the number of CD3\(^+\) CD4\(^+\) T lymphocytes in ileum and PPS of piglets**

The ileum and PPS of piglets were sliced, and the number of CD3\(^+\) CD4\(^+\) T lymphocytes in the intestine and on PPS was detected by immunofluorescence. The results are shown in Figure 9 and Figure 10. The number of CD3\(^+\) CD4\(^+\) T lymphocytes on the ileum of LGG group was higher than that of PBS group, and the number of CD3\(^+\) CD4\(^+\) T lymphocytes on PPS of LGG group was also higher than that of PBS group.

**Effect of LGG on differentiation of CD3\(^+\) CD4\(^+\) T lymphocytes in piglets**

The CD3\(^+\) CD4\(^+\) T lymphocytes of piglets were separated by flow cytometry and RNA was extracted. The gene expression of CD3\(^+\) CD4\(^+\) T lymphocyte transcription factor was detected by fluorescence quantitative PCR. The results are shown in Figure 11. The expression of T-bet gene in Th1 type cells was significantly higher than that in PBS group (P < 0.001). There was no difference in the expression of GATA gene between Th2 type cells and PBS group (P > 0.05). The expression of ror gene in Th17 type cells was significantly higher than that in PBS group (P > 0.05). The expression of Foxp3 + in Treg group was significantly lower than that in Shengli group (P < 0.001).
Figure 9: The number of ileal CD4+ CD3+ T lymphocytes in piglets fed LGG at 15d.

Figure 10: The number of PPs CD4+ CD3+ T lymphocytes in piglets fed LGG at 15d.

Discussion

LGG can survive in the digestive tract environment of animals, and has stable colonization in human and animal intestines, regulation of intestinal flora, prevention and treatment of diarrhea, elimination of toxins, and improvement of body immunity. It can be seen from these points that *Lactobacillus rhamnosus* is widely used in human body, but the research on livestock and poultry aquatic products is relatively blank, so the in-depth research on *Lactobacillus rhamnosus* is still in constant exploration [14]. At present, it has been proved that LGG can regulate t-lymph of piglets, but it is not clear which component of LGG plays a role. It has been reported that P40 can activate Akt, inhibit the apoptosis of epithelial cells induced by cytokines, and promote the growth of human and mouse colonic epithelial cells and cultured colonic explants. Research shows. P40 protein can indirectly act on B cells through MSIE cells, increase the production of IgA+ B cells, and promote the production of IgA in IgA+ B cells. Therefore, we hypothesized whether P40 is also the main protein regulating T lymphocyte in piglets. Researchers have developed a pectin/zein hydrogel containing P40 using P40 protein.
to deliver P40 to the small intestine and colon. The hydrogel was then given to mice. It was found that the weight of the mice fed with P40 hydrogel increased significantly from second days to twenty-first days after birth, and the intestinal function was fully developed, including intestinal epithelial cell proliferation, differentiation and tight junction formation, and early IgA production in mice. Therefore, we purified P40 protein, stimulated piglet lymphocytes with P40, and detected the number of T lymphocytes. Firstly, we successfully purified P40 protein. After stimulation, the results were consistent with the hypothesis that P40 was the main protein regulating T lymphocyte in piglets. It was found for the first time that P40, a secreted protein of LGG, is the main component of T lymphocyte regulation in piglets. At present, there are few reports on this aspect, which can lay the foundation for the follow-up research.

A large number of experiments have proved that probiotics can mediate human cellular immunity. Cellular immunity refers to the release of cytokines by T lymphocytes to mediate inflammation, so as to play an immune response. It was found that LGG can induce human T cells and dendritic cells to secrete cytokines [15]. It has been found that LGG can improve the proliferation of human T cells and increase the number of T cells [16]. Studies have shown that LGG can increase the number of T lymphocytes, thereby reducing the recurrence of human colitis [17]. In this experiment, we used LGG to administrate piglets in advance, and detected the number of T-lymphocytes in each lymph node and intestinal tract, as well as the differentiation of T-lymphocytes mediated by LGG. According to the analysis of the experimental results, there is no difference between the number of T-lymphocytes in MLN mediated by LGG and PBS group. We analyzed the reasons. As a secondary lymphoid organ, mln circulates continuously in the whole blood and tissue fluid of T-lymphocytes. Because the ligand integrin released by the homing receptor Ma

![Figure 11: Expression of CD3+ CD4+ T lymphocyte transcription factor gene in piglet MLN with LGG at 15d.](image-url)
release a large number of ligands. In the following experiments, there was no significant difference in the number of T lymphocytes between the experimental group and the control group. So, in the next experiment, we will continue to verify this problem. We also analyzed the changes of the number of T-lymphocytes in other lymph nodes. From the results, we can know that LGG can induce the increase of the number of T-lymphocytes no matter in PP node or intestinal lamina propria, which also verified the results of previous studies. LGG has the function of inducing the proliferation of T-lymphocytes and increasing the number of T-lymphocytes, no matter in human or piglet. It also provides a reference for the development of new Lactobacillus vaccine. T lymphocytes in the intestine can release a large number of cytokines to mediate intestinal inflammation. Because of this advantage of Lactobacillus, the new Lactobacillus vaccine will be paid more and more attention.

At the same time, we also tested the effect of LGG on the differentiation of CD4+ T lymphocytes. CD3+ CD4+ T lymphocytes mainly differentiate into Th1 type T lymphocytes, Th2 type T lymphocytes, Th17 type T lymphocytes and Treg type T lymphocytes, but these four types of T lymphocytes also play a role of mutual restriction [18]. The main function of Th1 cells is to stimulate the differentiation of monocytes to cause local inflammatory response, and to participate in the immune response caused by intracellular pathogens. Th2 cells are mainly involved in humoral immunity and limit parasite infection. Th17 is mainly involved in the infection of some pathogens. The main function of Treg cells is immune suppression. It is generally believed that the imbalance of differentiation of these four types of T lymphocytes is also a cause of intestinal damage and diarrhea [19]. Therefore, in order to be more accurate, CD3+ CD4+ T lymphocytes are sorted out. From our results, we can see that Th2 and Th17 are relatively stable. The number of Th1 cells was higher than that of Treg cells. But it also remains relatively stable. To sum up, LGG can promote the differentiation of CD3+ CD4+ T cells to Th1 cells, so as to resist the infection of pathogenic bacteria. This study is the first in China to study the effect of LGG on the differentiation of T lymphocyte in early weaned piglets, which provides a reference for the development of a new type of Lactobacillus vaccine.

Conclusion

LGG can promote the proliferation of lymphocytes in early weaned piglets, increase the number of CD3+ CD4+ T lymphocytes in PPS and LP, and CD4+ CD8+ T lymphocytes in IEL. LGG can promote the expression of T-bet transcription factor of CD3+ CD4+ T lymphocytes in piglet MLN, and promote the differentiation of CD3+ CD4+ T lymphocytes into Th1 cells.

The P40 protein secreted by LGG is the main effector protein of regulating T-lymphocytes in piglets. P40 can stimulate the proliferation of T-lymphocytes and increase the number of CD3+ CD4+ T-lymphocytes.

Declarations

Ethics approval and consent to participate: This study was carried out in agreement with the principles established by Jilin Agriculture University Changchun China and guide for the use of laboratory and care animals and all experimental protocols were approved by a Jilin Agriculture University (No. JLAU08201007).

Consent for Publication

Not applicable

Availability of data and materials: Will be provided after acceptance.

Competing Interests

Author 1 declares that she has no conflict of interest
Author 2 declares that he has no conflict of interest
Author 3 declares that he has no conflict of interest
Author 4 declares that he has no conflict of interest
Author 5 declares that she has no conflict of interest.

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Authors’ Contributions

Author 1 SMS -searching data, wrote manuscript and acted as corresponding author
Author 2 BF -searching data, wrote manuscript and acted as corresponding author
Author 3 WY -editing manuscript
Author 4 GY- editing the manuscript and supervision of the manuscript
Author 5 CW- editing the manuscript and supervision of the manuscript
All authors have read and approved the manuscript.

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