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Growth Promotion and Economic Benefits of the Probiotic *Lactiplantibacillus plantarum* in Calves

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Abstract: Objectives: Various measures have been attempted to prevent infectious diseases in calves, such as environmental improvement and vaccine administration. Probiotics are commonly used to improve the body condition of newborn calves and prevent disease. In our previous research, *Lactiplantibacillus plantarum* RGU-LP1 (LP1) suppressed the expression of inflammatory cytokines in PBMCs of cattle fed it in the diet. In this study, we evaluated the effect of LP1 on the weights and number of treatments of the calves. **Methods**: Twenty-six one-week-old Holstein bull calves were divided into two groups (thirteen each), the LP1 group (LP1-treated) and the CN group (no LP1 fed), and tested as follows. The LP1 group was fed lyophilized LP1 (10^9 CFU/head/day) in milk replacer for 40 days. The CN group was fed the same diet only. Calves were followed for 63 days. The average treatment costs for the LP1 during the period were recorded. Feces and blood were collected from each calf during this period. Feces were examined for gut microbiota, and blood for immune assay and cytokine gene expression. **Results**: The LP1-treated group showed a decrease in disease incidence and an increase in body weights compared to controls. The average treatment cost during the observation period was significantly reduced compared to the CN group. The expression of *TGFβ* and *IL10*, inhibitory cytokines of inflammation, was significantly increased. The simultaneous expression of this set of inhibitory molecules resulted in low serum IL1β levels during the growth period. **Conclusions**: The Th1-type cytokine *IFNγ* was also significantly increased in LP1-treated calves. By reducing the amount of disease treatments and increasing dairy gain, LP1 is effective in preventing infectious diseases in calves. In addition, the increase in *IFNγ* by LP1 indicates improved Th1-type immunity in calves. These results show that LP1 has effects on the regulated inflammatory response and growth of calves.

Keywords: calf; probiotics; microbiota; cytokines; infectious diseases

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1. Introduction

Calves are born without gammaglobulin, and the immune system of newborns is immature [1,2]; therefore, they are more susceptible to pathogens, which affects their growth [3–5]. Therefore, on-farm disease prevention measures are taken by improving the rearing environment and vaccination program [6–9]. Probiotics are one of the most widely used preventive measures against infectious diseases in animal production. In 1989, Fuller defined probiotics as "live, orally available microorganisms that have a beneficial effect on the host by improving the intestinal microflora" [10]. In 1998, Salminen et al. [11] redefined probiotics as "live microorganisms or foods containing them that exert a health effect when administered to the host in appropriate amounts". In addition, the effects of probiotics vary depending on the strain administered and, in particular, the immunostimulatory effects of Lactobacillus probiotics vary depending on the strain [12]. *Lacticaseitobacillus casei* activates Th1-type immune responses [13] and *Limosilactobacillus reuteri* activates Th2-type immune responses [14], and some strains of bacteria themselves induce Th1-type immune responses

and others induce Th2-type immune responses. *Lactiplantibacillus plantarum* has been isolated from fermented foods such as pickles, and examples of its functional properties include preventing obesity and insulin resistance [15], improving immune activity and reducing stress [16], as well as anti-inflammatory effects [17].

The *Lactiplantibacillus plantarum* RGU-LP1 strain (LP1) used in this study has been reported to control inflammation in adult cattle [18]. The strain has also been shown to reduce lung inflammation and regulate the production of inflammatory cytokines in a mouse model of asthma [19].

It is expected to support health management by improving the immune balance and gut microbiota of newborn calves with immature immunomodulatory mechanisms. Therefore, the aim of this study was to evaluate the effects of feeding LP1 to newborn calves on growth and immune function and to compare the economic costs of calf rearing.

2. Materials and Methods

2.1. Experimental Animals

Holstein calves (male, 1 week old) were obtained from a cattle market in Hokkaido, Japan, and were housed individually in calf hutches to avoid direct contact. All calves were clinically observed for a 5-day acclimation period, and healthy calves were used for the experiment. In all, 26 healthy calves were used in the trial. The calves were divided into two groups of 13 calves each in LP1-treated (using the LP1 strain, a functional *L. plantarum*) and LP1-non-treated (CN) groups. All experiments were conducted in accordance with the animal care guidelines of the Scientific Feed Laboratory Co., Ltd. in accordance with the Basic Guidelines for the Conduct of Animal Experiments in Research Institutions, etc., issued by the Ministry of Education, Culture, Sports, Science and Technology (approval number: 078-2021-1).

2.2. Probiotics

The functional lactic acid bacterium *Lactiplantibacillus plantarum* RGU LP1 strain (LP1) was used in the dosing study. LP1 was processed into lyophilized granules for administration, and adjusted to a bacterial count of 10^8 CFU/g at the Scientific Feed Research Institute Ltd. (Tokyo, Japan). Calves were fed milk replacer (Milk Dash, National Federation of Agricultural Cooperative Associations) daily in the morning and evening. LP1 was administered by mixing 10 g of LP1 powder per calf in the morning (daily dose 10^9 CFU/calf/day) with the milk replacer. Control calves received the same amount of milk replacer only.

2.3. Study Protocol for Probiotic Feeding

LP1 was administered after a 5-day acclimatization period following the arrival of the calves from the livestock market. The probiotic treatment period lasted 40 days and all calves were housed in calf hutches. After the dosing period, the calves were housed in open pens and observed for 63 days from the start of LP1 feeding (Figure 1).

2.4. Fecal and Blood Samples

Fecal samples were collected at 0, 14 and 28 days after the start of LP1 administration for the analysis of gut microflora. Blood samples were taken at 0, 14, 28, 49 and 63 days after the start of treatment to measure the health check and serum cytokines and to assess vaccine antibody titers. Peripheral blood mononuclear cells (PBMCs) were analyzed for cytokine gene expression on days 28 and 63.

2.5. Clinical Observation

At follow-up, fecal scores, daily weight gain, number of treatments and total treatment costs were evaluated. Fecal scores were assessed during the rearing period of the calves. Fecal score was defined as score 1 for normal feces, score 2 for soft feces and score 3 for watery faces. Daily gain was calculated as the average daily gain per animal based on body weight at the time of initiation and at the end of the treatment period. The number of

treatments was performed for 63 days from the start of dosing to the end of observation, and the number of treatments and total treatment cost (JPY) per animal in each group were calculated based on the number of treatments received per individual during this period and the number of treatment points, excluding the cost of home visits.

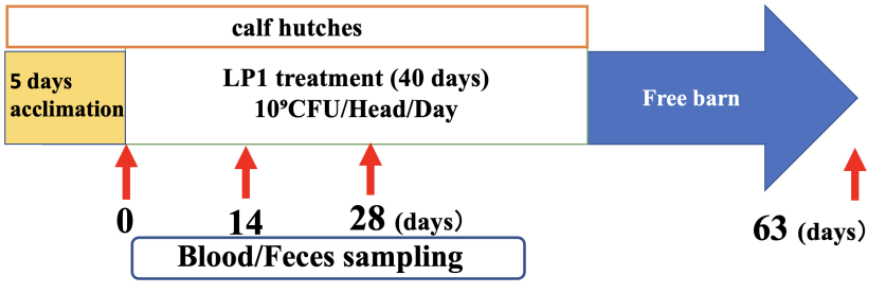

Figure 1. Overview of the LP1 feeding schedule. The calves were clinically healthy at one week of age, and the calves were housed individually in calf hutches and kept acclimatized for 5 days. Calves were divided into LP1-treated (LP1) and control (CN) groups for the study. Calves received LP1 (10^9 CFU/head/day) with milk replacer for 40 days, while the control group received equal amounts of the same milk replacer. After the completion of treatment, each calf was moved to a free barn and kept under the same feeding management. Red arrows indicate blood or fecal sampling.

2.6. Intestinal Bacteria Culture

Intestinal bacteria were analyzed on selective media for four genera (*Lactobacillus* sp., *Bifidobacterium* sp., *Clostridium* sp. and *Coliform* bacteria) and total anaerobic bacteria. Fecal samples were diluted 10-fold in sterile phosphate-buffered saline (PBS) and then added to BBL™ LBS agar (Becton, Dickinson, Franklin Lakes, NJ, USA), BS agar, DHL agar (Nissui Pharmaceuticals, Osaka, Japan) and modified GAM agar. BS agar medium was prepared by adding BS additive to BL agar medium (Nissui Pharmaceutical Co., Ltd., Hokkaido, Japan) and sterilizing it at 121 °C for 15 min according to the manufacturer's instructions, and then adding horse defibrinated blood to reach a final concentration of 10%. GAM agar medium was prepared by adding Bacto™ Agar (Becton, Dickinson, Franklin Lakes, NJ, USA) to GAM liquid medium (Nissui) to a final concentration of 1.5% and sterilizing at 115 °C for 15 min. The DHL agar medium was incubated under aerobic conditions at 37 °C for 24 h, and the LBS agar, BS agar, CW agar and GAM agar medium were incubated under anaerobic conditions at 37 °C for 48 h. The genera and species of bacteria identified on the selective media used in this study were Lactobacillus for LBS agar, Bifidobacterium for BS agar, *E. coli* and hydrogen sulfide producing bacteria for DHL agar and Clostridium for CW agar. The CFU/g of each bacterial species in 1 g of feces was calculated by measuring the colonies detected after incubation on selective media.

2.7. Serum Cytokine

Bovine serum interleukin-1β (IL1β) and interleukin-6 (IL6) levels were measured on days 0, 14, 28 and 63 after LP1 administration. Serum IL1β levels were determined using the Bovine IL1β ELISA Kit (Invitrogen, Carlsbad, CA, USA) and serum IL6 levels were determined using the Bovine IL6 ELISA Reagent Kit (Invitrogen, Carlsbad, CA, USA). The assay was performed according to the kit protocol. Quantitative values for each cytokine were determined by calculation based on the kit standards.

2.8. Peripheral Blood Mononuclear Cell (PBMC) Isolation

EDTA-supplemented blood was separated from the PBMC layer by Ficoll–Conrey (specific gravity 1.086) density gradient centrifugation. Centrifugation was performed at 2500 rpm for 20 min. After the collection of the PBMC layer, the cells were washed with PBS and resuspended in serum-free RPMI 1640 with 5% FCS to adjust the cell count to 2×10^6 cells/mL. The PBMCs were used for the following cytokine gene expression assay.

2.9. Measurement of Cytokine Gene Expression

PBMCs from each calf were stimulated with lipopolysaccharide (LPS O111: B4; Sigma-Aldrich Japan G.K., Osaka, Japan) and incubated with LPS (final concentration: 5 μg/mL in RPMI 1640 medium) for 6 h at 37 °C, with a 5% CO_2 gas incubator. Unstimulated PBMCs were used as controls. After the reaction, samples were subjected to RNA extraction using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the instructions. The extracted RNA was cDNA, synthesized using ReverTra Ace (Toyobo, Gifu, Japan) oligo(dT)20 primers according to the kit manual. *Interleukin-2* (*IL2*), *interleukin-5* (*IL5*), *interleukin-10* (*IL10*), *interleukin-12* (*IL12*), *interferon-γ* (*IFNγ*), *transforming growth factor-β* (*TGFβ*) and *housekeeping genes* (*GAPDH*) cytokine gene expression was quantified by Rotor-Gene (Qiagen, Hilden, Germany) using KOD SYBR qPCR Mix (Toyobo, Shiga, Japan) (Supplementary Table S1). The gene expression was normalized to *GAPDH* expression and calculated by ΔΔCT analysis.

2.10. Statistical Analysis

Statistical significance was determined using R Studio (R version 4.0.3) with Student's *t*-test, Welch's *t*-test, Mann–Whitney u-test, the Steel–Dwass method and Turkey Honest Significant Differences (Turkey HSD) as multiple comparison tests, and those showing $p < 0.05$ were considered as significant differences. Significant differences are indicated in the figure as follows: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. The numerical values of the results are presented as mean + standard error. For parametric tests, 95% confidence intervals for the difference in means between the LP1 group and the CN group were calculated using Excel (Microsoft® Excel® for Microsoft 365 MSO).

3. Results

3.1. Clinical Follow-Up and Medical Costs

No calves died during the observation period, but there were individuals who became ill during their stay in the calf hatches. Some animals in the LP1 group had transient soft stools during the administration period, but none had diarrhea. The fecal score was 1.31 ± 0.03 for the LP1 group and 1.29 ± 0.03 for the CN group, with no significant difference in fecal scores ($p = 0.918$, Figure 2A). Daily gain average was also 0.60 kg/head/day in the LP1 group and 0.58 kg/head/day in the CN group, with slightly better gain in the LP1 group ($p = 0.518$, Figure 2B and Supplementary Table S2). In terms of number of treatments, one animal with pneumonia in the LP1 group received treatment on day 13 of lactation. In contrast, six cows in the CN group were treated (one with pneumonia and five with diarrhea, Supplementary Table S3). The total number of treatments, converted per animal, is shown in Figure 2C. The number of treatments was 0.07 per cow in the LP1 group and 1.4 per cow in the CN group, significantly lower in the LP1 group ($p = 0.02$), and the total treatment cost per cow was JPY 126.6 in the LP1 group and JPY 2259.2 in the CN group, indicating that LP1 treatment reduced total medical costs by JPY 2133 (about USD 15.1, EUR 13.6, $p = 0.02$, Figure 2D).

3.2. Comparison of Gut Bacteria Counts

The number of bacteria of each genus detected in the feces on days 0, 14 and 28 after treatment is expressed as colony forming units/g (CFU/g, Figure 3).

For the *Lactobacillus* species, the number of bacteria in the LP1 group remained at $\log_{10}$ 7.32 to $\log_{10}$ 7.28 CFU/g from day 0 to 28 after treatment, while in the CN group it decreased from $\log_{10}$ 7.83 to $\log_{10}$ 6.20 CFU/g from day 0 to 28 after treatment. The decrease was significantly lower at day 28 compared to the LP1 group ($p = 0.009$, Figure 3A). For *Clostridium* species, the LP1 group showed an increase from $\log_{10}$ 4.69 CFU/g to $\log_{10}$ 5.60 CFU/g from day 0 to 28 after treatment, while the CN group showed a decrease from $\log_{10}$ 5.36 to $\log_{10}$ 4.54 CFU/g from day 14 to 28 after treatment. And the difference between the two groups changed significantly on day 28 ($p = 0.00005$, Figure 3B). Other

Bifidobacterium species, coliform groups and total anaerobic bacteria counts did not differ significantly between groups during the observation period (Figure 3C–E).

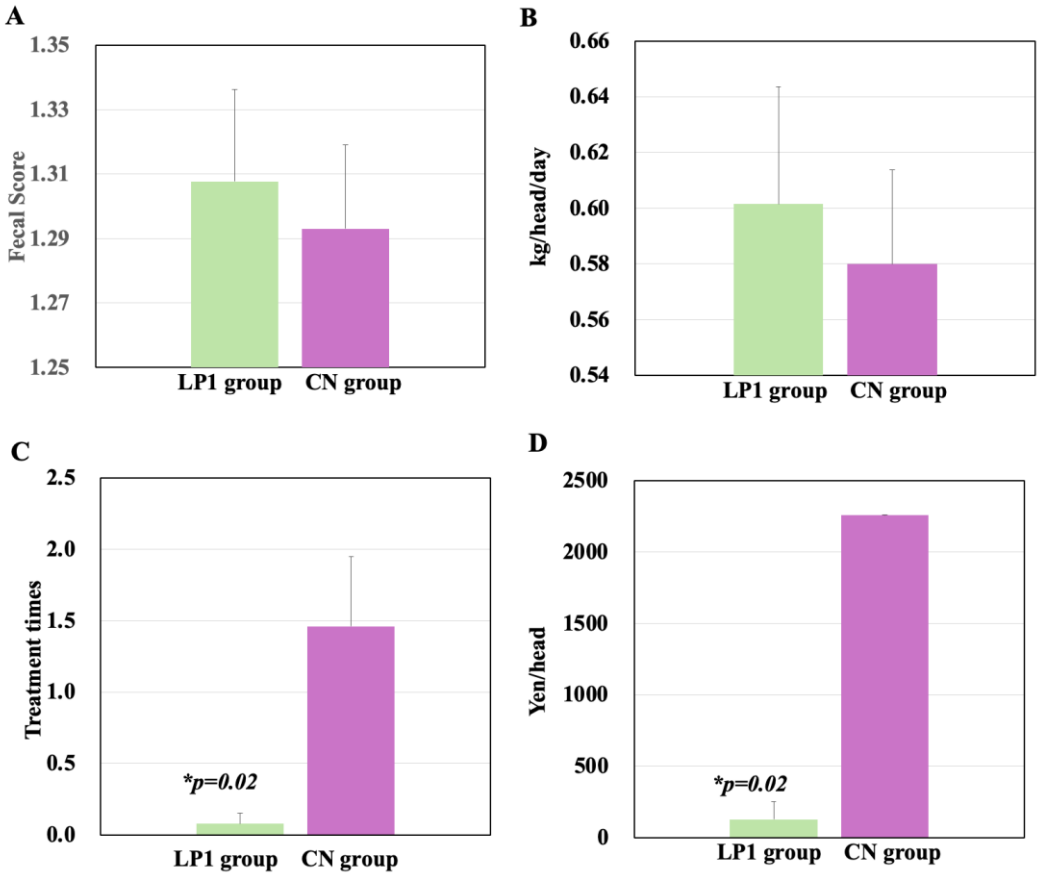

Figure 2. Comparison of health status, number of treatments and cost of treatment with and without LP1 feeding. This figure shows the results of group comparisons with and without LP1 administration for four items (LP1, LP1-treated group; CN, LP1-untreated control group). The comparison items are as follows: (**A**): fecal score, (**B**): daily gain, (**C**): number of treatments, and (**D**): total treatment cost. A Mann–Whitney u-test was performed for statistical analysis between the two groups, LP1 and CN (n = 13 each). Values are presented as mean + standard error, indicating a decrease in the number of treatments and total medical costs with LP1 treatment. LP1: *L. plantarum* LP1-treated group, CN: control group. * $p < 0.05$.

3.3. ILβ and IL6 in Serum

Serum IL1β and IL6 concentrations were measured by indirect sandwich ELISA. At 0, 14 and 28 days after LP1 treatment, IL1β concentration trends were significantly decreased in the LP1 group compared to the CN group at 14 and 28 days after treatment (p = 0.004) and at 14 days (p = 0.008) (Figure 4A). The trend in IL6 concentration was not significantly different between groups before treatment, but was significantly lower in the LP1 group compared to the CN group at 14 and 28 days after treatment (p = 0.0004) at 14 and 28 days (p = 0.02, Figure 4B). In Figure 4C, Serum IL1β concentration 3 weeks after the end of LP1 treatment and the concentration of IL1β in the serum is shown; statistical analysis between the two groups confirmed a significant difference by Welch's t test (** $p < 0.01$).

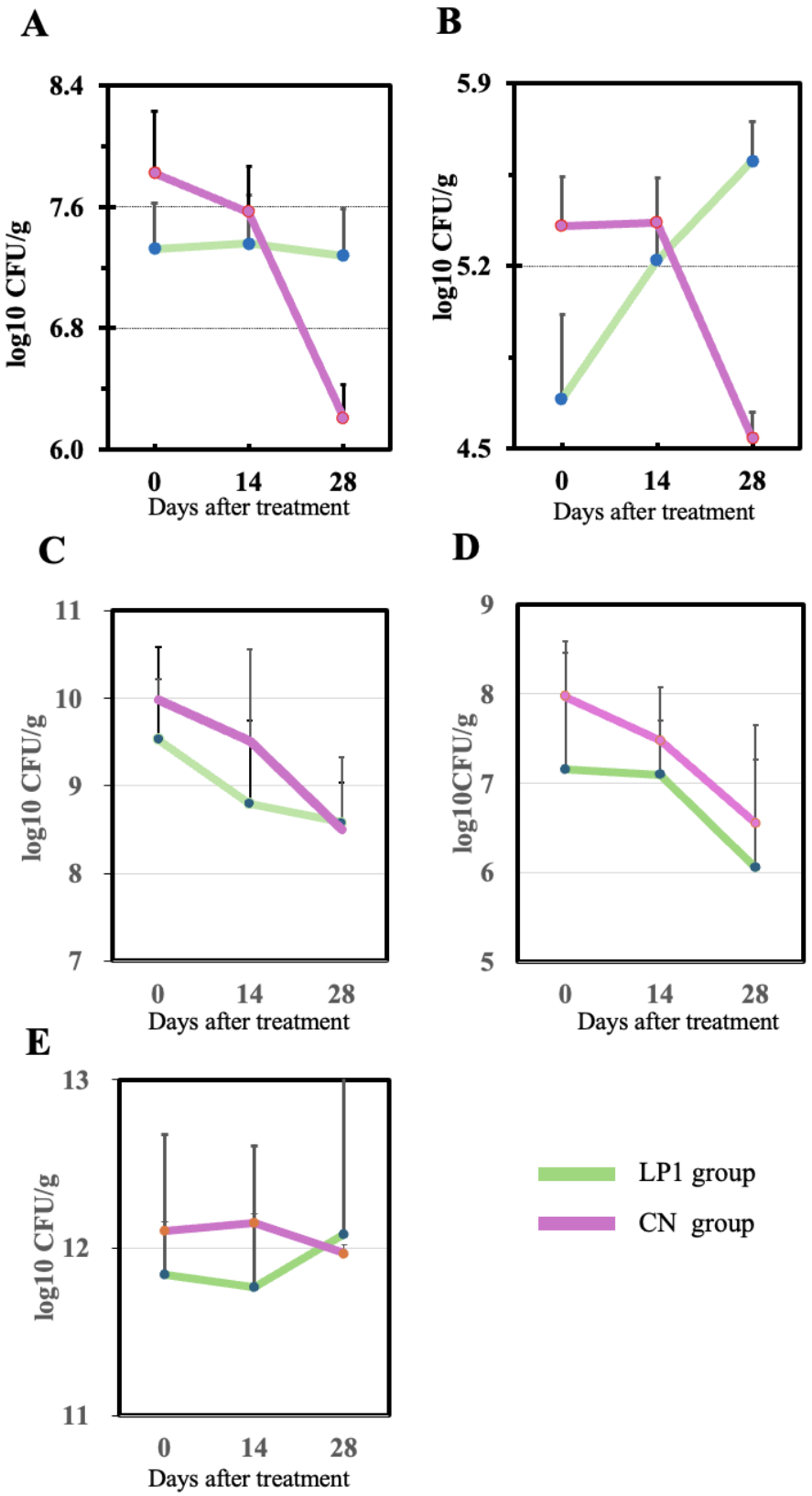

Figure 3. Intestinal bacterial changes with LP1 feeding. Fecal samples from calves in each group were cultured on selective media: (**A**): *Lactobacillus* sp.; (**B**): *Clostridium* sp.; (**C**): *Bifidobacterium* sp.; (**D**): *Coliform* group; (**E**): total anaerobic bacteria count. Horizontal axis indicates days after LP1 administration (0, 14, 28 days). Number of calves in each group: LP1 (n = 13) and CN (n = 13). Welch's t test was used for intergroup analysis. The numbers on the x-axis indicate the days after treatment.

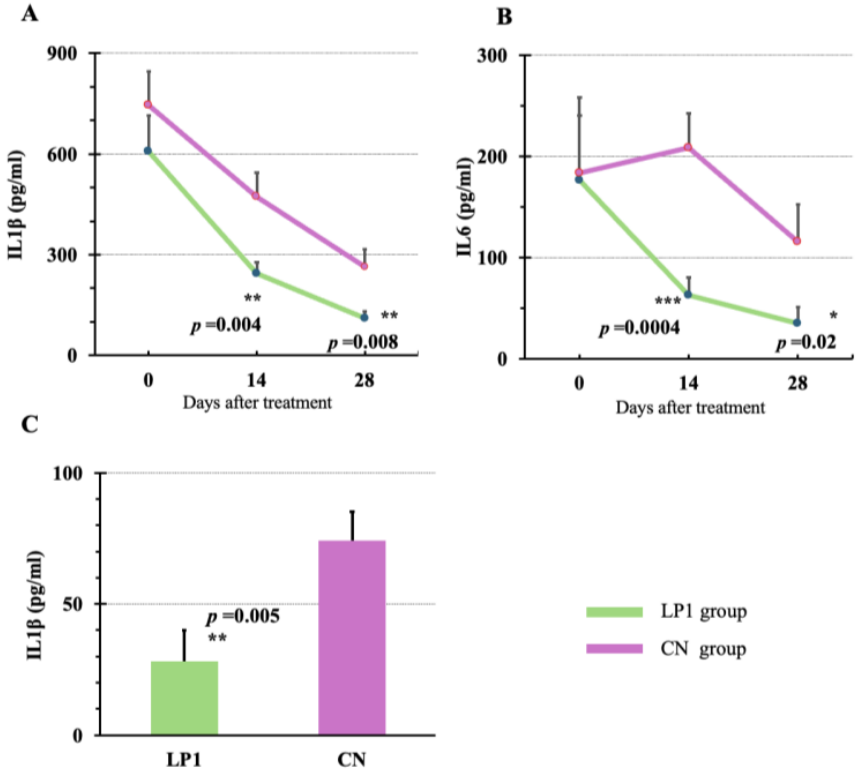

Figure 4. Comparison of serum IL1β and IL6 concentrations. Serum IL1β and IL6 concentrations quantified by ELISA; the mean and standard deviation are shown in each graph as follows: (**A**): serum IL1β; (**B**): serum IL6. X axis indicates number of days after LP1 treatment (0, 14, 28 days). Number of calves per group: LP1 (n = 13); CN (n = 13). Student's t test was used to analyze between two groups at each sampling point. (**C**): Serum IL1β concentration 3 weeks after the end of LP1 feeding. Concentration of IL1β in serum is shown; statistical analysis between the two groups confirmed a significant difference with Welch's t test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). LP1: *L. plantarum* LP1-treated group, CN: control group.

3.4. Cytokine Gene Expression in PBMC 28 Days After LP1 Treatment

The gene expression of each cytokine was examined in LPS-stimulated PBMC 28 days after treatment. The cytokines examined were *IL2*, *IL5*, *IL10*, *TGFβ* and *IFNγ*. *GAPDH* expression was normalized as a housekeeping gene. *IL10* expression was significantly higher in the LP1 group (17.88 vs. 2.51 in the CN group (p = 0.0001, 95CI (7.326, 23.402)), while *TGFβ* showed a difference in mean values, but not significantly (p = 0.396; 95CI (−2.406, 3.106), Figure 5A,B). Furthermore, in the LP1 and CN groups, *IL2* expression was significantly higher in the LP1 group, with 5.29 versus 1.47 in the CN group (p = 0.0002, 95CI (1.877, 5.763). Similarly, *IFNγ* expression was significantly higher in the LP1 group, 26.85 versus 2.65 in the CN group (p = 0.0063; 95CI (4.657, 43.743), Figure 5C,D). On the other hand, *IL5* expression was 2.3 in the CN group versus 1.34 in the LP1 group, showing a trend towards higher gene expression in the CN group, but no significant difference was found (p = 0.084; 95CI (−0.108, 2.028), Figure 5E).

3.5. Serum Cytokine Levels on Day 63 After Lp1 Feeding

To evaluate the effect of LP1 treatment after the completion of the treatment, serum IL1β and IL6 were measured on day 63 after the start of treatment. Serum IL1β was significantly lower in the LP1 group at 28.12 pg/mL compared to 74.04 pg/mL in the CN group (p = 0.005; Figure 4C) when the concentrations of both groups were compared. In addition, serum IL6 was not detected in each group on the same day.

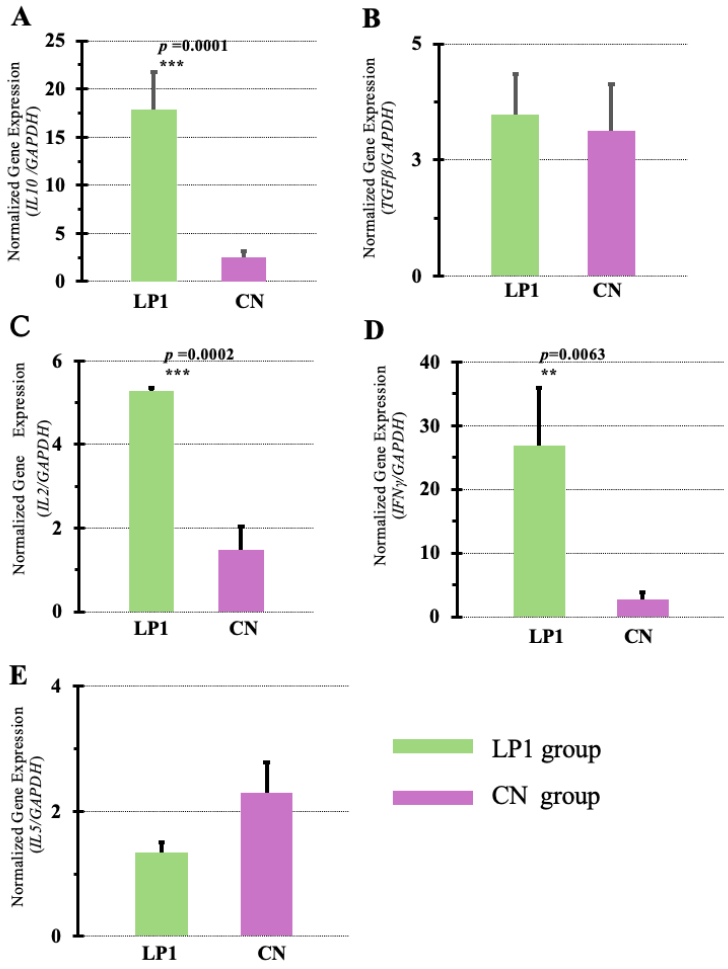

Figure 5. Cytokine gene expression in LPS-stimulated PBMCs 28 days after LP1 feeding. Cytokine gene expression in LPS-stimulated PBMCs 28 days after LP1 treatment. Each cytokine gene expression was normalized by GAPDH expression, and the mean and standard deviation are shown in each graph as follows: (**A**): *IL10*; (**B**): *TGFβ*; (**C**): *IL2*; (**D**): *IFNγ*; (**E**): *IL5*. Columns show LP1: *L. plantarum* LP1-treated group (n = 13) and CN: (n = 13) control group. Statistical analysis between the two groups was based on F-test, Student's t-test and Welch's t-test. In the statistical analysis of the comparison group, less than 5% of the differences were significant. ** $p < 0.01$, *** $p < 0.001$.

3.6. Comparison of Cytokine Expression in PBMCs 3 Weeks After the End of LP1 Treatment

The gene expression of each cytokine was examined using PBMCs obtained from calves 63 days after LP1 administration (3 weeks after the end of LP1 treatment). The cytokines analyzed were *IL2*, *IL10*, *IL12*, *TGFβ* and *IFNγ*. *GAPDH* expression was standardized as a housekeeping gene. *IL10* expression was significantly higher (p = 0.01, 95CI (0.288, 2.700)) in the CN group (0.65 vs. 2.14 in the LP1 group); *TGFβ* expression was significantly higher (0.358) in the CN group vs. 0.991 in the LP1 group, and a difference in means was found (p = 0.09, Figure 6A,B). *IL2* expression was 1.418 in the LP1 group and 1.413 in the CN group with no difference between groups (p = 0.5); *IL12* expression was significantly higher in the LP1 group, at 1.89 compared to 0.46 in the CN group (p = 0.02, 95CI (0.053, 2.807)). *IFNγ* expression was 0.13 in the CN group versus 0.36 in the LP1 group, with a difference in the mean but not a significant one (p = 0.07, Figure 6C–E).

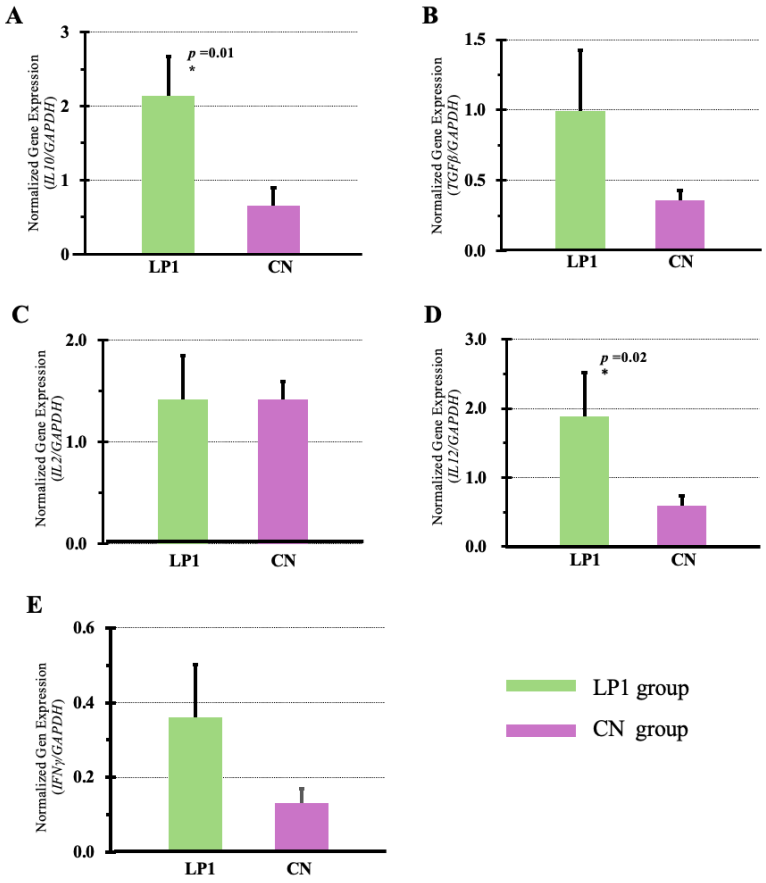

Figure 6. Cytokine gene expression in PBMCs 3 weeks after the end of LP1 treatment. Each cytokine gene expression was normalized by *GAPDH* expression, and the mean and standard deviation are shown in each graph as follows: (**A**): *IL10*; (**B**): *TGFβ*; (**C**): *IL2*; (**D**): *IL12*; (**E**): *IFNγ*. Columns show LP1: *L. plantarum* LP1-treated group and CN: control group. Statistical analysis between LP1 (LP1-treated group, n = 13) and CN (control group, n = 13) groups was based on F-test, Student's t-test and Welch's t-test. In the statistical analysis of the comparison group, less than 5% of the differences were significant. * $p < 0.05$.

4. Discussion

The immature immune systems of newborn mammals can render them vulnerable to disease as maternal antibodies decline. Probiotics are widely known to contribute to livestock health and productivity [20]. It has previously been reported that the LP1 strain, a functional *Lactiplantibacillus plantarum*, can modulate inflammation in adult cattle [18], and this study showed that it enhances immune function in calves and confers resistance to infections such as pneumonia and diarrhea. The efficacy of treatment with LP1 was evaluated by examining changes in gut microbes and cytokine expression in PBMC, and by comparing growth rate, disease incidence and the cost of treatment between groups.

Diarrhea is one of the leading causes of mortality in newborn calves, and most of this occurs in the first month of life [21,22]. Diarrhea is often caused by rotavirus or coronavirus, in combination with bacterial species. Diarrhea and pneumonia are the most costly diseases in calf management and cause significant economic losses by affecting subsequent growth [23,24]. Preventing these newborn calf diseases and reducing the number of treatments will reduce health care costs and bring economic benefits.

The gut microbiota is closely related to a calf's immune function and stimulates the development of immune function and the control of allergies [25]. The gut microbiota changes significantly with disease and antibiotic use and has been reported to be affected in calves [5,26,27]. In our study, the LP1 treatment of calves maintained stable *Lactobacillus* spp. and increased *Clostridium* spp. The increase in *Clostridium* spp. may be partly due to

the LP1 strain stabilizing bacterial diversity by maintaining calf health. *Lactobacillus* spp. produces lactic acid, a type of volatile fatty acid, and volatile fatty acids have functions in the intestinal tract such as immune regulation, increasing peristalsis and aiding nutrient absorption [28].

Butyric acid bacteria, such as *Clostridium butyricum*, were selectively cultured on the CW agar medium used in this study [29]; it has been reported that butyric acid-producing bacteria induce regulatory T cells via the production of butyrate, a volatile fatty acid, in the intestinal tract. These regulatory T cells (hereafter referred to as Treg) produce IL10 and are involved in immune regulation [30]. It is therefore suggested that the increase in butyrate-producing bacteria increased Tregs and regulated immune function. There are anti-inflammatory and immunomodulatory effects of lactic acid bacteria-derived short-chain fatty acids (SCFAs). In the periphery, particularly in the gut-pulmonary axis, a reduction in SCFA-induced lung inflammation may also be a factor in the results of this study [31,32]. These findings suggest that LP1 administration may improve the gut microbiota and act on gut and lung immune function. Some *Lactobacillus* spp. produce SCFAs, which induce regulatory T cells [33], suggesting that they play a role in controlling inflammation. In this study, the direct effect of LP1 and changes in the intestinal microbiota that act to control inflammation may also be a factor.

A comparison of serum cytokine levels showed that LP1 treatment significantly reduced both IL1β and IL6 inflammatory cytokines. On the other hand, a significant upregulation of *IL2*, *IL10* and *IFNγ* gene expression was observed in LPS-stimulated PBMCs. The excessive production of pro-inflammatory cytokines causes biological damage such as fever and anorexia, whereas anti-inflammatory cytokines such as IL10 regulate inflammatory responses by controlling the production of pro-inflammatory cytokines [34–36]. While previous studies have shown that LP1 suppresses inflammatory cytokines and increases *IL10* expression in adult cattle [18], the present study showed that LP1 also has an inflammation-modulating effect in calves.

The results of this study suggest that LP1 regulates the expression of inflammatory cytokines in response to LPS stimulation, accompanied by *IL10* expression, to low levels appropriate for immune activation in calves, thereby controlling excessive inflammation. Thus, LP1 is expected to regulate exaggerated immune responses to antigenic stimuli, such as pathogens, and promote appropriate cytokine expression by modulating the calf's exaggerated immune response to infection. This is expected to reduce disease exacerbation in newborn calves and shorten treatment time. In addition, this effect is sustained for approximately 3 weeks after the cessation of feeding, making it a valuable probiotic for livestock management. A number of papers have reported the efficacy of Lactobacillus strains in controlling inflammation, and the potential of this species is under development [37–40]. This will have applications in anti-pathogenic properties and the appropriate use of antimicrobial agents.

This study showed that feeding LP1 to calves stabilizes intestinal microbiota diversity and regulates immune function to control excessive inflammatory response, resulting in economic benefits for disease management. In addition, LP1 can induce appropriate cytokine induction in response to microbial antigens such as LPS, and this is expected to suppress disease progression in newborn calves and reduce treatment time. Although the functional components derived from LP1 could not be elucidated in this study, we will investigate the biological effects of cell wall components such as peptidoglycan and fatty acids, extracellular polysaccharides, lactic acid bacteria metabolites, SCFA and nucleic acids on immunoregulatory functions.

5. Conclusions

The administration of functional *Lactiplantibacillus plantarum* (LP1) to newborn calves for several weeks is expected to not only improve the intestinal microbiota but also control excessive inflammatory responses. This LP1 strain contributes to the economic cost of

rearing by reducing the incidence of disease during the newborn period and reducing the cost of treatment by enhancing the innate immunity of the treated calves.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijtm4040041/s1, Supplementary Table S1: Primers for cytokine gene expression; Table S2: Body weight change and dairy gain of calves after the 40 days LP1 treatment; Table S3: Overview of drugs for disease treatment and dates of disease.

Author Contributions: K.O. Sample collection and preliminary processing, investigation, molecular biology analysis, writing—original draft, data curation, software application; S.T.: sample collection and preliminary processing, investigation, molecular biology analysis, methodology, investigation, data curation, software application and formal analysis; C.I.: sample collection and preliminary processing, methodology, formal analysis, preliminary processing; T.T.: methodology, visualization, investigation, formal analysis, software, validation, sample collection and preliminary processing; K.H.: conceptualization, methodology, validation, supervision, writing—review and editing, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The study was conducted with the Scientific Feed Laboratory, Co. Ltd. providing granulated powder processing of lactic acid bacteria for use in the study sample.

References

1. Cortese, V.S. Neonatal Immunology. *Vet. Clin. N. Am. Food Anim. Pract.* **2009**, *25*, 221–227. [CrossRef] [PubMed]
2. Cuttance, E.; Laven, R. Perinatal mortality risk factors in dairy calves. *Vet. J.* **2019**, *253*, 105394. [CrossRef] [PubMed]
3. Abe, F.; Ishibashi, N.; Shimamura, S. Effect of Administration of Bifidobacteria and Lactic Acid Bacteria to Newborn Calves and Piglets. *J. Dairy Sci.* **1995**, *78*, 2838–2846. [CrossRef] [PubMed]
4. Kimura, N.; Yoshikane, M.; Kobayashi, A.; Mitsuoka, T. An Application of Dried Bifidobacteria Preparation to Scouring Animals. *Bifid-Microflora* **1983**, *2*, 41–54. [CrossRef]
5. Takino, T.; Kato-Mori, Y.; Orihashi, T.; Hagiwara, K. Rotavirus Infection Transiently Affects Intestinal Microbiome Composition in Newborn Calves. *J. Vet. Med. Res.* **2018**, *5*, 205–450.
6. Matty, J.M.; Reddout, C.; Adams, J.; Major, M.; Lalman, D.; Biggs, R.; Salak-Johnson, J.L.; Beck, P.A. The Effects of Respiratory Vaccine Type and Timing on Antibody Titers, Immunoglobulins, and Growth Performance in Pre- and Post-Weaned Beef Calves. *Vet. Sci.* **2023**, *10*, 37. [CrossRef]
7. Sandelin, A.; Härtel, H.; Seppä-Lassila, L.; Kaartinen, L.; Rautala, H.; Soveri, T.; Simojoki, H. Field trial to evaluate the effect of an intranasal respiratory vaccine protocol on bovine respiratory disease incidence and growth in a commercial calf rearing unit. *BMC Vet. Res.* **2020**, *16*, 73. [CrossRef]
8. Erickson, N.E.N.; Berenik, A.; Lardner, H.; Lacoste, S.; Campbell, J.; Gow, S.; Waldner, C.; Ellis, J. Evaluation of bovine respiratory syncytial virus (BRSV) and bovine herpesvirus (BHV) specific antibody responses between heterologous and homologous prime-boost vaccinated western Canadian beef calves. *Can. Vet. J.* **2021**, *62*, 37–44. [PubMed]
9. Dubrovsky, S.A.; Van Eenennaam, A.L.; Aly, S.S.; Karle, B.M.; Rossitto, P.V.; Overton, M.W.; Lehenbauer, T.W.; Fadel, J.G. Preweaning cost of bovine respiratory disease (BRD) and cost-benefit of implementation of preventative measures in calves on California dairies: The BRD 10K study. *J. Dairy Sci.* **2020**, *103*, 1583–1597. [CrossRef]
10. Fuller, R. Probiotics in man and animals. *J. Appl. Bacteriol.* **1989**, *66*, 365–378.
11. Salminen, S.; Bouley, C.; Boutron, M.-C.; Cummings, J.H.; Franck, A.; Gibson, G.R.; Isolauri, E.; Moreau, M.-C.; Roberfroid, M.; Rowland, I. Functional food science and gastrointestinal physiology and function. *Br. J. Nutr.* **1998**, *80* (Suppl. S1), S147–S171. [CrossRef] [PubMed]
12. Fang, H.; Elina, T.; Heikki, A.; Seppo, S. Modulation of humoral immune response through probiotic intake. *FEMS Immunol. Med. Microbiol.* **2000**, *29*, 47–52. [CrossRef] [PubMed]

13. Perdigón, G.; Galdeano, C.M.; Valdez, J.; Medici, M. Interaction of lactic acid bacteria with the gut immune system. *Eur. J. Clin. Nutr.* **2002**, *56*, S21–S26. [CrossRef] [PubMed]
14. Mustafa, S.M.; Chua, L.S.; El-Enshasy, H.A.; Majid, F.A.A.; Hanapi, S.Z.; Malik, R.A. Effect of temperature and pH on the probiotication of *Punica granatum* juice using *Lactobacillus* species. *J. Food Biochem.* **2019**, *43*, e12805. [CrossRef]
15. Okubo, T.; Takemura, N.; Yoshida, A.; Sonoyama, K. KK/Ta Mice Administered Lactobacillus plantarum Strain No. 14 Have Lower Adiposity and Higher Insulin Sensitivity. *Biosci. Microbiota, Food Health* **2013**, *32*, 93–100. [CrossRef]
16. Nishimura, M.; Ohkawara, T.; Tetsuka, K.; Kawasaki, Y.; Nakagawa, R.; Satoh, H.; Sato, Y.; Nishihira, J. Effects of yogurt containing Lactobacillus plantarum HOKKAIDO on immune function and stress markers. *J. Tradit. Complement. Med.* **2015**, *6*, 275–280. [CrossRef]
17. Park, M.-K.; Ngo, V.; Kwon, Y.-M.; Lee, Y.-T.; Yoo, S.; Cho, Y.-H.; Hong, S.-M.; Hwang, H.S.; Ko, E.-J.; Jung, Y.-J.; et al. Lactobacillus plantarum DK119 as a Probiotic Confers Protection against Influenza Virus by Modulating Innate Immunity. *PLoS ONE* **2013**, *8*, e75368. [CrossRef]
18. Chida, S.; Sakamoto, M.; Takino, T.; Kawamoto, S.; Hagiwara, K. Changes in immune system and intestinal bacteria of cows during the transition period. *Vet. Anim. Sci.* **2021**, *14*, 100222. [CrossRef]
19. Alawneh, J.I.; Barreto, M.O.; Moore, R.J.; Soust, M.; Al-Harbi, H.; James, A.S.; Krishnan, D.; Olchowy, T.W. Systematic review of an intervention: The use of probiotics to improve health and productivity of calves. *Prev. Vet. Med.* **2020**, *183*, 105147. [CrossRef]
20. Kishida, S.; Kato-Mori, Y.; Okamoto, M.; Hagiwara, K. Anti-inflammatory effect a specific Lactiplantibacillus plantarum in an ovalbumin-induced asthma model. *Microbiol. Immunol.* **2022**, *66*, 442–452. [CrossRef]
21. Urie, N.J.; Lombard, J.E.; Shivley, C.B.; Kopral, C.A.; Adams, A.E.; Earleywine, T.J.; Olson, J.D.; Garry, F.B. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. *J. Dairy Sci.* **2018**, *101*, 9229–9244. [CrossRef] [PubMed]
22. Osorio, J.S. Gut health, stress, and immunity in neonatal dairy calves: The host side of host-pathogen interactions. *J. Anim. Sci. Biotechnol.* **2020**, *11*, 1–15. [CrossRef] [PubMed]
23. Cho, Y.-I.; Yoon, K.-J. An overview of calf diarrhea—Infectious etiology, diagnosis, and intervention. *J. Vet. Sci.* **2014**, *15*, 1–17. [CrossRef] [PubMed]
24. Peel, D.S. The Effect of Market Forces on Bovine Respiratory Disease. *Vet. Clin. N. Am. Food Anim. Pract.* **2020**, *36*, 497–508. [CrossRef]
25. Hua, X.; Goedert, J.J.; Pu, A.; Yu, G.; Shi, J. Allergy associations with the adult fecal microbiota: Analysis of the American Gut Project. *EBioMedicine* **2016**, *3*, 172–179. [CrossRef]
26. Keijser, B.J.F.; Agamennone, V.; Broek, T.J.v.D.; Caspers, M.; van de Braak, A.; Bomers, R.; Havekes, M.; Schoen, E.; van Baak, M.; Mioch, D.; et al. Dose-dependent impact of oxytetracycline on the veal calf microbiome and resistome. *BMC Genom.* **2019**, *20*, 65. [CrossRef]
27. Mitsuoka, T. Establishment of intestinal bacteriology. *Biosci. Microbiota Food Health* **2014**, *33*, 99–116. [CrossRef]
28. Sivieri, K.; Morales, M.L.V.; Adorno, M.A.T.; Sakamoto, I.K.; Saad, S.M.I.; Rossi, E.A. Lactobacillus acidophilus CRL 1014 improved " gut health" in the SHIME® reactor. *BMC Gastroenterol.* **2013**, *13*, 100. [CrossRef]
29. Klopp, R.N.; Franco, J.F.H.; Hogenesch, H.; Dennis, T.S.; Cowles, K.E.; Boerman, J.P. Effect of medium-chain fatty acids on growth, health, and immune response of dairy calves. *J. Dairy Sci.* **2022**, *105*, 7738–7749. [CrossRef]
30. Kanai, T.; Mikami, Y.; Hayashi, A. A breakthrough in probiotics: Clostridium butyricum regulates gut homeostasis and anti-inflammatory response in inflammatory bowel disease. *J. Gastroenterol.* **2015**, *50*, 928–939. [CrossRef]
31. Maslowski, K.M.; Vieira, A.T.; Ng, A.; Kranich, J.; Sierro, F.; Yu, D.; Schilter, H.C.; Rolph, M.S.; Mackay, F.; Artis, D.; et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* **2009**, *461*, 1282–1286. [CrossRef]
32. Vaughan, A.; Frazer, Z.A.; Hansbro, P.M.; Yang, I.A. COPD and the gut-lung axis: The therapeutic potential of fibre. *J. Thorac. Dis.* **2019**, *11*, S2173–S2180. [CrossRef] [PubMed]
33. Arpaia, N.; Campbell, C.; Fan, X.; Dikiy, S.; Van Der Veeken, J.; DeRoos, P.; Liu, H.; Cross, J.R.; Pfeffer, K.; Coffer, P.J.; et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **2013**, *504*, 451–455. [CrossRef] [PubMed]
34. Beheshtipour, J.; Raeeszadeh, M. Evaluation of interleukin-10 (IL-10) and pro-inflammatory cytokines profile in calves naturally infected with neonatal calf diarrhea syndrome. *Arch. Razi Inst.* **2020**, *75*, 213–218. [CrossRef] [PubMed]
35. Saraiva, M.; Vieira, P.; O'garra, A. Biology and therapeutic potential of interleukin-10. *J. Exp. Med.* **2019**, *217*, e20190418. [CrossRef] [PubMed]
36. Sheil, B.; MacSharry, J.; O'Callaghan, L.; O'Riordan, A.; Waters, A.; Morgan, J.; Collins, J.K.; O'Mahony, L.; Shanahan, F. Role of interleukin (IL-10) in probiotic-mediated immune modulation: An assessment in wild-type and IL-10 knock-out mice. *Clin. Exp. Immunol.* **2006**, *144*, 273–280. [CrossRef]
37. Lu, Q.; Guo, Y.; Yang, G.; Cui, L.; Wu, Z.; Zeng, X.; Pan, D.; Cai, Z. Structure and Anti-Inflammation Potential of Lipoteichoic Acids Isolated from *Lactobacillus* Strains. *Foods* **2022**, *11*, 1610. [CrossRef]
38. Yang, J.; Cho, H.; Gil, M.; Kim, K.E. Anti-Inflammation and Anti-Melanogenic Effects of Maca Root Extracts Fermented Using *Lactobacillus* Strains. *Antioxidants* **2023**, *12*, 798. [CrossRef]

39. Kang, Y.; Kang, X.; Yang, H.; Liu, H.; Yang, X.; Liu, Q.; Tian, H.; Xue, Y.; Ren, P.; Kuang, X.; et al. Lactobacillus acidophilus ameliorates obesity in mice through modulation of gut microbiota dysbiosis and intestinal permeability. *Pharmacol. Res.* **2022**, *175*, 106020. [CrossRef]
40. Rastogi, S.; Singh, A. Gut microbiome and human health: Exploring how the probiotic genus Lactobacillus modulate immune responses. *Front. Pharmacol.* **2022**, *13*, 1042189. [CrossRef]

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