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Milk-based Drinks containing Lactic Acid Bacteria on Gut Dysbiosis and Birth Weight from Undernourished Pregnant Rats

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Abstract

Lactic Acid Bacteria (LAB) can be an alternative intervention to help improve pregnancy outcomes. This study aimed to examine the effects of milk-based drinks (MBD) containing a single strain, namely *Lactobacillus casei Shirota strain*, and MBD containing multiple strains, namely *Lactobacillus delbrueckii subsp. Bulgaricus*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, and *Streptococcus thermophilus* to improve gut dysbiosis and offspring birthweight in malnourished pregnant rats, conducted at the Experimental Animal Laboratory from 2020 to 2021. The sample consisted of female rats that were induced with malnutrition and mated. Twenty-four female Sprague Dawley rats, aged 7-8 weeks, weighing 150-250 grams, were randomly divided into one negative control group (A0) and three malnourished groups. The malnourished groups consisted of the positive control group (A1), MBD-LcS group (A2), and MBD-multi strain group (A3). The intervention was administered for 19 days. Data was analyzed using ANOVA. Results showed that administering MBDs significantly affected total coliform bacteria ($p<0.05$). Moreover, it significantly affected the offspring's birth weight. The highest birth weight was found in the A3 group (5.18±0.88 g). Overall, prodiving MBDs containing beneficial bacteria significantly improved total coliform bacteria and pregnancy outcomes. Thus, it can help reduce nutritional and health problems in the future.

Introduction

Chronic Energy Deficiency (CED) in women of childbearing age or pregnant women remains a major nutritional and health problem in Indonesia. Data from the 2023 Indonesian Health Survey shows that as many as 17% of pregnant women are at risk of CED (Kemenkes RI, 2024). Malnutrition during pregnancy can increase the risk of anemia, hypertension, miscarriage, and even fetal death. Furthermore, CED in pregnant women can result in low birth weight (LBW) babies and intrauterine growth retardation, which ultimately leads to impaired quality of life and long-term healthcare costs (Khayati *et al.*, 2025; Oktriyanto *et al.*, 2022; Simbolon *et al.*, 2021; Wati *et al.*, 2022). Several studies showed that this condition can be improved by providing local foods or *lactic acid bacteria* (Basri *et al.*, 2025; Li *et al.*, 2024; Nahdyah *et al.*, 2025; Shi *et al.*, 2026).

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Under normal conditions, the gut microbiota is dominated by beneficial bacteria such as LAB, which play a role in maintaining intestinal homeostasis, higher nutrient absorption, and maintaining the integrity of the intestinal barrier (Kumar *et al*., 2025). In CED, deficiency of nutrient intake, particularly fiber, protein, and micronutrients, can disrupt the availability of substrates for bacterial growth. This leads to a decrease in the LAB population and an increase in opportunistic bacteria such as coliforms, resulting in an imbalance of microbiota composition, called dysbiosis. Dysbiosis can reduce the production of short-chain fatty acids (SCFAs), which play a crucial role in maintaining intestinal mucosal health and energy metabolism (Herrnreiter *et al*., 2025). This condition can increase intestinal permeability (leaky gut), allowing the translocation of endotoxins such as lipopolysaccharide (LPS) into the systemic circulation. Increased LPS triggers chronic low-grade inflammation in pregnant women, which negatively impacts nutrient metabolism and placental function, thus affecting the transfer of nutrients and oxygen from maternal to fetus (Abu & Roy, 2025).

Improving dysbiosis through the administration of LAB is a promising approach to improve maternal health and pregnancy outcomes. LAB are known to modulate the composition of the gut microbiota through several key mechanisms, including competition with pathogenic bacteria for nutrients and adhesion sites on the intestinal mucosa, production of organic acids such as lactic acid that lower the pH of the intestinal environment, and secretion of antimicrobial compounds such as bacteriocins that can inhibit the growth of coliform bacteria and other pathogens (D. Li *et al*., 2024; Shi *et al*., 2026). Through these mechanisms, LAB contributes to increasing the dominance of beneficial bacteria and suppressing the growth of opportunistic bacteria, thereby helping to restore the balance of the gut microbiota (D. Li *et al*., 2024; Pino *et al*., 2025; Shi *et al*., 2026).

Although probiotics, particularly LAB, have been widely reported to play a role in maintaining human health and can help nutritional transfer from maternal to fetus, the direct link between maternal gut microbiota modulation and pregnancy outcomes remains incompletely understood, particularly in malnourished conditions. Furthermore, most studies have focused on single-strain probiotics, resulting in limited comparative evidence regarding the effectiveness of single-strain and multi-strain probiotics in ameliorating dysbiosis in malnourished conditions. Currently, in the Indonesian market, there are two popular milk-based drinks (MBDs) containing LAB. The first has a single strain of LAB, namely *Lactobacillus casei* Shirota strain (LcS), and the latter contain a mixture of four LAB (*Lactobacillus rhamnosus, Lactobacillus paracasei, Lactobacillus delbrueckii* subp. *Bulgaricus,* and *Streptococcus thermopillus*). Therefore, this study was conducted to evaluate and compare the effects of administering MBDs containing single-strain and MBDs containing multi-strain on improving the gut dysbiosis in malnourished pregnant rats and their effect on the offspring's birth weight, thereby providing a more comprehensive understanding of the role of the gut microbiota as an intervention target in improving pregnancy outcomes. .

Method

This study used a randomized controlled trial (RCT), conducted in vivo at the Experimental Animal Laboratory, Faculty of Veterinary Medicine, IPB University. We used 8-10 weeks-old female *Sprague Dawley* (*Rattus norvegicus)* rats with an initial body weight of approximately 150-200g, obtained from a laboratory animal care facility. Prior to treatment, the animals were acclimatized for 7 days. After acclimatization, the rats were then randomly divided into two groups: the negative control group (A0), with normal conditions, and the malnourished groups. All research procedures were conducted in accordance with animal welfare principles and received approval from the Animal Ethics Commission, Faculty of Veterinary Medicine, IPB University, No. 025/KEH/SKE/2020.

Induction of malnutrition before conception was given by a low protein and restriction diet (50%). The results of malnutrition induction can be seen in the decreasing body weight of the rats by 8-22%,

which is categorized as mild (Merino-Sanjuán *et al*., 2011). The undernourished rats were then randomly divided into three groups: the positive control group (A1), the MBDs-LcS (A2), and the MBDs-Multi Strain (A3). The detailed procedure was already published by Nurwati *et al*. (2021). Mating is performed when female rats are in the final phase of proestrous and beginning estrous. Determination of the estrous cycle was done by (vaginal smear) (Pantier *et al*., 2019). The mating process lasted overnight by mixing male and female rats in the same cage, from 6:00 pm to 6:00 am. At 06.00 am, pregnancy was checked using the vaginal plug or vaginal smear method (Boğa Kuru & Kuru, 2023). Female rats were declared pregnant on day 0 if the vaginal plug revealed a yellowish-white mass blocking the rats' vagina. To convince the results, a vaginal smear was performed to determine the presence of sperm using a microscope.

The intervention group (A2 and A3) was administered orally using oral gavage daily throughout pregnancy as much as 1.5 mL/day; the A0 and A1 received mineral water with the same amount. The MBDs-LcS group received MBDs containing LcS ($7.2x10^7$ CFU/mL), and the MBDs-multi strain group received a mixture of LAB ($1.2x10^9$ CFU/mL). The microbiology assessment was done by collecting the rats' feces before and at the end of pregnancy. Samples were serially diluted using sterile physiological saline, then inoculated onto selective media: *Man Rogosa Sharp* (MRS) agar for LAB and *violet red bile agar* (VRBA) for coliform bacteria. At 19 days of pregnancy, rats were sacrificed for data collection. The birth weight of rats' offspring was measured immediately after birth (postnatal day 0) using a digital scale with an accuracy of 0.01 grams. Data analysis was performed using statistical software. The total of LAB and coliform bacteria, body weight, and food intake of rats were presented as mean±standard deviation. Main analysis using one-way analysis of variance (ANOVA) followed up with Duncan Multiple Range Test (DMRT) post-hoc test. A p-value <0.05 was considered statistically significant.

Results and Discussions

Feed conversion efficiency (FCE) scores were also calculated to assess the ratio of body weight gain and food intake in each group. The higher the FCE score, the more efficient the feed provided was in increasing the rats' body weight. Table 1 shows the amount of feed consumed, rat weight gain, and FCE scores during the intervention.

Based on Table 1, the total food intake of the groups was not significantly different. However, there was a trend toward increased feed consumption from A0 (343.00 ± 48.05 g) to A3 (407.00 ± 31.95 g), but this variation was not statistically significant (indicated by the superscript letter a for all groups). Conversely, body weight gain showed a significant difference between groups ($p<0.039$). Groups A1 (80.00 ± 10.58 g) and A3 (81.00 ± 8.54 g)

TABLE 1. Feed Consumption, Rats' Weight Gain, and Feed Conversion Efficiency (FCE) during the Intervention

Groups	Total Food Intake (g)	Weight Gain (g)	FCE (%) (BW/intake x 100%)
A0	343.00 ± 48.05[a]	70.67 ± 5.43[a,b]	20.60 ± 2.05[b]
A1	396.67 ± 20.00[a]	80.00 ± 10.58[b]	20.17 ± 2.67[b]
A2	400.50 ± 38.89[a]	63.00 ± 9.76[a]	15.73 ± 2.12[a]
A3	407.00 ± 31.95[a]	81.00 ± 8.54[b]	20.10 ± 3.55[b]
	p [1)] = 0.09	p [1)] = 0.039*	p [1)] = 0.042*

(Primary data, 2021)

*Mean ± SD for n=6. *p* value <0.05 is statistically significant for One-way ANOVA test

TABLE 2. The Average Number of LAB in Pregnant Rats Before and After Intervention

Groups	Before (log CFU/g)	After (log CFU/g)	Δ (log CFU/g)
A0	7.22 ± 0.48[a]	8.56 ± 1.25[a]	1.33 ± 1.52[a]
A1	7.41 ± 0.48[a]	8.58 ± 0.82[a]	1.16 ± 1.23[a]
A2	7.89 ± 0.72[a]	7.53 ± 1.57[a]	-0.45 ± 0.85[a]
A3	7.89 ± 0.40[a]	8.38 ± 0.77[a]	0.49 ± 0.96[a]
	p [1)] = 0.166	p [1)] = 0.587	p [1)] = 0.200

(Primary data, 2021)

*Mean ± SD for n=6. *p* value <0.05 is statistically significant for One-way ANOVA test

had higher weight gain and were significantly different from group A2 (63.00 ± 9.76 g), while group A0 (70.67 ± 5.43 g), as a negative control group, was intermediate (indicated by the letters a,b). For feed conversion efficiency (FCE), a significant difference was also found between groups (*p<0.042*). Group A0 (20.60 ± 2.05%), A1 (20.17 ± 2.67), and A3 (20.10 ± 3.55) had higher FCE values and did not differ from each other but were significantly higher than group A2 (15.73 ± 2.12). Overall, these results indicate that intervention of MBDs in groups A3 tended to increase weight gain and feed utilization efficiency compared to group A2, without significantly affecting feed consumption. Table 2 represents the average LAB counts in log CFU/mL for each group, including the changes that occurred after intervention.

Table 2 presents the mean ± standard deviation (SD) of LAB counts in Log CFU/g as well as the change (**Δ**) in all groups. Pre-intervention conditions showed no significant difference in LAB counts among groups (*p*=0.166). Mean values ranged from 7.22±0.48 log CFU/g (A0) to 7.89±0.72 log CFU/g (A2 and A3), indicating that the initial microbiota was relatively homogeneous across all groups. After intervention, LAB counts also showed no significant differences between groups (*p*=0.587). However, descriptively, there was an increase in the number of LAB in groups A0 (8.56 ± 1.25 log CFU/g), A1 (8.58 ± 0.82 log CFU/g), and A3 (8.38 ± 0.77 log CFU/g), while group A2 showed a decreasing trend (7.53 ± 1.57 log CFU/g). Analysis of the change in LAB count also showed no significant difference between groups (*p*=0.200). Numerically, groups A0 (1.33 ± 1.52 log CFU/g) and A1 (1.16 ± 1.23 log CFU/g) experienced a greater increase in LAB counts than group A3, while A2 experienced a decrease in total LAB count. However, none of these differences were statistically significant. Overall, these results indicate that the intervention was unable to produce significant changes in the number of LAB in pregnant rats, although there was a trend towards an increase in some treatment groups.

Coliform bacteria generally indicate the presence of pathogens in the human or animal digestive tract, which can negatively impact human health. Table 3 shows the average number of coliform bacteria in pregnant rats before and after intervention.

TABLE 3. The Average Number of Coliform Bacteria in Pregnant Rats Before and After Intervention

Groups	Before (log CFU/g)	After (log CFU/g)	Δ (log CFU/g)
A0	2.32 ± 0.91[a]	3.48 ± 1.22[a]	1.16 ± 1.73[b]
A1	7.04 ± 0.74[b]	3.92 ± 1.95[a]	-4.89 ± 2.43[a]
A2	6.25 ± 0.89[b]	2.67 ± 2.21[a]	-2.98 ± 2.01[a]
A3	4.02 ± 0.41[a]	4.53 ± 2.13[a]	0.53 ± 2.37[b]
	p [1)] = 0.000*	p [1)] = 0.507	p [1)] = 0.008*

(Primary data, 2021)

*Mean ± SD for n=6. **p* value <0.05 is statistically significant for One-way ANOVA test

Table 3 presents the mean ± standard deviation (SD) of coliform counts (in log CFU/g) in pregnant rats before and after the intervention, as well as the change (Δ) in the four treatment groups (A0, A1, A2, A3), with a sample size of n=6 for each group. Pre-intervention conditions showed a significant difference in coliform counts between groups (p=0.000). Groups A1 (7.04 ± 0.74 log CFU/g) and A2 (6.25 ± 0.89 log CFU/g) had significantly higher coliform counts than groups A0 (2.32 ± 0.91 log CFU/g) and A3 (4.02 ± 0.41 log CFU/g), as indicated by the different letter notations (a and b). This indicates that the initial coliform microbiota was not homogeneous between groups. After the intervention, coliform counts did not show significant differences between groups (p=0.507). Mean values ranged from 2.67 ± 2.21 log CFU/g (A2) to 4.53 ± 2.13 log CFU/g (A3), indicating that the intervention tended to reduce and/or balance coliform counts across all groups. Analysis of changes in coliform counts (Δ) revealed significant differences between groups (p=0.008). Groups A1 (-4.89 ± 2.43 log CFU/g) and A2 (-2.98 ± 2.01 log CFU/g) experienced a greater and significantly different reduction in coliform counts compared to groups A0 (1.16 ± 1.73 log CFU/g) and A3 (0.53 ± 2.37 log CFU/g), which actually showed a trend towards an increase in coliform counts. Overall, these results indicate that the intervention was effective in reducing coliform counts, especially in groups A1 and A2, although no significant differences were found between groups at the end of the intervention. Birth weight is an important indicator for assessing the health and early development of offspring in pregnant rats. Differences in birth weight may reflect the effects of interventions administered during the study. Therefore, Table 4 shows the average birth weight of rat offspring in all groups.

Table 4 shows a significant difference in birth weight between groups (p=0.000). Group A3 had the highest birth weight (5.18 ± 0.88 g) and significantly differed from the other groups. Groups A1 (4.90 ± 0.69 g) and A0 (4.59 ± 0.57 g) were in the intermediate level, while group A2 showed the lowest birth weight (3.86 ± 1.29 g). Based on the superscript notation, group A3 (c) was significantly different from groups A0 (b) and A2 (a), while group A1 (b, c) was in the intermediate position because it did not differ significantly from either group A0 or A3. Group A2 (a) had significantly lower birth weights than the other groups. Overall, these results indicate that intervention of MBDs containing multi-strain in group A3 had the most optimal impact on increasing the birth weight of rat offspring, while group A2 showed the lowest results. In this study, two MBDs were given to the intervention groups. The former contains LcS and the latter contains four strains. Induction of malnutrition caused alteration in gut microbiota, and it can be seen in Table 3 that total coliform bacteria was significantly different among groups before intervention. After the MBD application, the coliform bacteria were not significantly different among groups. These results are in line with a study conducted by Radwan (2021) that administering the probiotic *Lactobacillus delbrueckii* and *Lactobacillus fermentum* can reduce the *E.coli* in rats and its toxic effect. Other research conducted by Li *et al.* (2025) showed that short-term *L.paracasei* 207-27 administration improves the gut microbiota composition.

Table 4. The Average Offspring Birth Weight Among Groups

Groups	Birth weight (g)
A0	4.59 ± 0.57^{b}
A1	$4.90 \pm 0.69^{b,c}$
A2	3.86 ± 1.29^{a}
A3	5.18 ± 0.88^{c}
	$p^{1)} = 0.000^{*}$

(Primary data, 2021)

*Mean ± SD for n=6. **p* value <0.05 is statistically significant for One-way ANOVA test

Gut dysbiosis is defined as an imbalance in the composition and function of the microbiota, which leads to disruption of gastrointestinal homeostasis and an increased risk of various metabolic and inflammatory diseases (Stiemsma & Michels, 2018). Triggers for dysbiosis include a low-fiber diet, antibiotic consumption, and exposure to environmental stress and even malnutrition (Mukherjee *et al*., 2025). Fermented food-based interventions have been reported as an effective nutritional approach in modulating the gut microbiota toward a state of eubiosis by providing live microorganisms and bioactive compounds (Marco *et al*., 2021; Mukherjee *et al*., 2025; Stiemsma *et al*., 2020). The bioactive metabolites, such as SCFAs, act as essential energy sources for host cells and are reported to have a significant impact on host immune and metabolic functions. Suppressing the growth of coliform bacteria in the digestive tract can be achieved by increasing LAB. Lactic acid bacteria from fermented foods can survive and multiply in the digestive tract and compete with pathogenic bacteria. The adhesion of pathogenic bacteria to the digestive tract can be prevented if adequate mucus production is maintained. The acetate and lactate produced by lactic acid bacteria from fermented foods can decrease the luminal pH thus preventing the growth of pathogenic bacteria. Furthermore, LAB produces bacteriocins, an antimicrobial peptide that damages the cell membranes of pathogenic bacteria and competes directly (competitive exclusion) for adhesion sites and nutrients in the intestinal mucosa (Anjana & Tiwari, 2022; Gao *et al*., 2022; van Zyl *et al*., 2020).

The intestinal epithelium is directly connected to the lumen contents and intestinal flora. The intestinal barrier is a primary defense mechanism maintaining epithelial integrity and protecting organisms from the environment. Intestinal barrier defenses include a mucus layer, antimicrobial peptides, immunoglobulin A, and the epithelial junction adhesion complex. Gut microbial dysbiosis can downregulate tight junction proteins and muc2, (the major intestinal mucin) expression, culminating in compromised intestinal barrier integrity. Mucin is the main macromolecule of epithelial mucus. When barrier function is disrupted, bacteria and food antigens can enter the submucosa and induce an inflammatory response (Cao *et al*., 2017; Mukherjee *et al*., 2025; Neurath *et al*., 2025). Administration or consumption of probiotics maintains the intestinal epithelial barrier, increases intestinal mucosal adhesion, inhibits pathogen adhesion, competes with pathogenic bacteria, produces antimicrobial substances, and modulates the immune system (Gao *et al*., 2022; Tegegne & Kebede, 2022).

Some probiotic strains can help increase body weight, and others act as anti-obesity agents. The bacteria contained in group A3 drinks have been shown to increase body weight during pregnancy and influence the birth weight of rat offspring. A similar effect was seen in group A2, where LcS bacteria, as found in the study by Karimi *et al*. (2015) demonstrated that LcS played a role in helping reduce body weight. In line with weight gain results, the A2 groups had the lowest weight gain and FCE among other groups, which significantly affected the birth weight of rat offspring. Lactobacillus casei Shirota (LcS) is known to play a role in weight loss by modulating gut microbiota composition, reducing body fat mass, and improving lipid metabolism, including by lowering leptin levels and increasing adiponectin, as well as suppressing metabolic endotoxemia due to improved intestinal permeability. Furthermore, LcS increases the production of short-chain fatty acids (SCFAs) such as butyrate, which stimulate the secretion of the anorexigenic hormones glucagon-like-peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY), thereby suppressing appetite and reducing energy intake (Cervantes-Tolentino *et al*., 2020; Karimi *et al*., 2015). On the other hand, the A3 group given a mixture of LAB showed the highest weight gain and food intake compared to other groups. Higher FCE and weight gain in A3 groups resulted in higher birth weight of the offspring. In MBD given to A3 groups contains four strains which each strains has its own function. For example, study by Zhu *et al*. (2024) and Mârza *et al*. (2025) concluded that the increasing of SCFAs production caused by probiotic administration can stimulate epithelial cell proliferation and energy utilization in the gut. Furthermore, it

can enhance the digestion and absorption of nutrition, which is translated into higher daily weight gains and better feed efficiency.

Conclusions

Providing an MBD containing single and multi-strain LAB to pregnant rats has been shown to improve pregnancy outcomes. MBD intervention, particularly MBDs containing multiple strains, demonstrated better gut dysbiosis by decreasing the total of coliform bacteria. Furthermore, administration of MBDs containing multiple strains also results in higher weight gain and FCE presentation, thus affecting the heavier offspring's birth weight compared to MBDs containing LcS. Taken together, the presence study underscores the relevance of the administration of LAB as an intervention to improve pregnancy outcomes. However, these results have several limitations, such as being applicable only to medium malnutrition, not moderate or severe. Besides, the total of LAB in each MBD is variable because we used the MBDs that are already in the Indonesian market. Further research can formulate the MBDs with the same content of LAB per Ml.

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