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Short Communication

Probiotic-rich fermented milk from *Lactiplantibacillus plantarum* IIA-1A5: Effects on pregnancy health in the animal model

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Abstract

Previous studies of *Lactiplantibacillus plantarum* IIA-1A5 have shown its potential as a probiotic in modulating gut microbiota and providing health benefits; however, its effects during pregnancy remain underexplored. The aim of this study was to assess the safety of fermented milk enriched with *L. plantarum* IIA-IA5 in pregnant mice. An experimental study was conducted at Universitas Andalas, Padang, Indonesia. Two groups of pregnant mice (*Mus musculus* L.) were used, each with six mice. The control group received sterilized milk (10 mL/kg body weight (BW)), while the intervention group was given fermented milk containing *L. plantarum* IIA-1A5 (10^7 colony forming unit (CFU)/mL). The evaluated outcomes included maternal weight changes, fetal counts and measurements, and assessments of fetal morphology and skeletal development. Results indicated that the morphology of fetuses showed no significant differences between the control and intervention groups; both groups demonstrated normal development with no detected resorption sites, growth retardation, or hemorrhage. For skeletal development, both groups had the same bone counts, including frontal, parietal, intraparietal, exoccipital, supraoccipital, nasal, pre-maxilla, mandibular, thoracal, lumbar, sternum, and extremities. This study highlights that *L. plantarum* IIA-1A5-enriched fermented milk was safe, as no significant morphological or bone developmental abnormalities were observed, indicating its potential as a dietary supplement to support pregnancy health. However, further studies involving larger sample sizes may be needed to provide a more comprehensive assessment of its outcomes and safety.

Keywords: Lactic acid bacteria, pasteurized milk, safety, abnormality, pregnancy

Introduction

Lactic acid bacteria probiotics, found in products such as yogurt and kefir, are popular for their health benefits [1,2]. These functional foods support gut microbiota by enhancing adhesion and producing secondary metabolites, such as bacteriocins [3-5]. Bacteriocins are ribosomally synthesized peptides with broad-spectrum antimicrobial properties that disrupt cell membranes and cause cell death [6,7]. Despite their effectiveness as antimicrobial properties, bacteriocins have not replaced antibiotics, although combining bacteriocins with antibiotics has been suggested to enhance stability and synergistic effects [6,8-10].

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A previous study has successfully isolated *Lactiplantibacillus plantarum* IIA-1A5 from Ongole crossbreed cattle, demonstrating its potential as a probiotic [11]. *L. plantarum* IIA-1A5 produces plantaricin, a class II bacteriocin [12,13]. Plantaricin has shown anticancer activity against cervical, colon, and breast cancers [14,15]. The application of 0.3% plantaricin extended the storage time of meatballs to 20 hours at room temperature [16]. Despite its benefits, plantaricin safety for consumption remains untested.

Fermented milk products are valued for their probiotic benefits, with *L. plantarum* IIA-IA5 being a notable strain [17-19]. This lactic acid bacteria strain offers probiotic properties, digestive tract resistance, and potential therapeutic effects [17-19]. Given the growing interest in probiotics, evaluating the safety of fermented milk from *L. plantarum* IIA-IA5, especially during pregnancy, is crucial [17]. Consumption of fermented milk fortified with *L. plantarum* IIA-IA5 may benefit maternal and fetal health, but a thorough safety evaluation is necessary to ensure its safety in pregnant women.

Previous studies on *L. plantarum* strains, such as *L. plantarum* B [20], *L. plantarum* BJ0021 [21], and *L. plantarum* 299v [22], demonstrated beneficial effects in pregnant mice. *L. plantarum* IIA-IA5, known for its probiotic potential, also shows promise in modulating gut microbiota and providing health benefits [1,17]. However, its specific effects during pregnancy remain underexplored. The aim of this study was to assess the safety of fermented milk enriched with *L. plantarum* IIA-IA5 in pregnant mice, focusing on potential adverse effects on maternal health, pregnancy outcomes, and neonatal development, as well as exploring the underlying mechanisms of the probiotic strain's impact on the maternal-fetal unit.

Methods

Study design and setting

An experimental study was conducted from March to July 2023 at the Laboratories of Biotechnology and Pharmacology, Faculty of Animal Sciences, and Laboratory of Animal Research, Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia. The Laboratory of Biotechnology handled biological sample preparation, the Laboratory of Pharmacology assessed pharmacological effects, and the Laboratory of Animal Research ensured proper animal care under ethical standards. Two groups of pregnant mice (*Mus musculus* L.) were used, each group with six mice. The control group received sterilized milk (10 mL/kg body weight (BW)), while the intervention group received fermented milk containing *L. plantarum* IIA-1A5 (10^7 colony forming unit (CFU)/mL) each day. The evaluations included monitoring maternal weight changes, measuring and counting the fetuses, and assessing fetal morphology and skeletal development.

Sampling strategy and criteria

Healthy nulliparous female mice (18–20 grams; 4–5 weeks old) with normal behavior and stable weight (within 10% fluctuation) were included in the study. Mice exhibiting weight changes exceeding 10%, lacking pregnancy signs, or having severe health issues that could compromise the study were excluded. Following the Indonesian National Agency of Drug and Food Control guidelines [23], each group required five test animals. A total of 12 mice were used, equally divided into two groups of six. The sample size was selected to ensure sufficient statistical power for detecting differences between the control and intervention groups [24,25]. Pregnant mice were randomized, with odd numbers assigned to the control group and even numbers to the intervention group. Randomization was managed by a third party at the Laboratory of Animal Research, Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia, to ensure blinding of group allocation.

Fermented milk preparation

Fermented milk was prepared by inoculating sterilized milk with *L. plantarum* IIA-1A5 (5% volume) and incubating at 37°C for 18 hours, resulting in thickened milk. Fresh ferment was prepared daily. *L. plantarum* IIA-1A5, used in this study, was isolated by one of the authors (IIA). Before testing, two rounds of culturing were performed: the first to confirm homogeneity and

eliminate contaminants, and the second as a pre-test culture to ensure the bacteria were in the growth phase.

Animal husbandry and preparation

Mice were acclimatized for one week in plastic cages with wire lids, maintained at 23±3°C, a 12-hour light-dark cycle, and 50±10% humidity. They were fed a standard diet (75% carbohydrates, 16% protein, 55% fat, 3.6% calcium, and 0.4% phosphorus) with *ad libitum* access to water. Proper cage hygiene was ensured to maintain animal health and research integrity. Regular cleaning included removing soiled bedding, disinfecting cage surfaces, and replacing bedding. The cages were disinfected with appropriate chemicals to eliminate microorganisms and kept in well-ventilated areas to minimize harmful gases and odors. The bedding was frequently changed for moisture control, and waste was properly disposed of to prevent contamination. Routine health checks were conducted to monitor hygiene-related issues. Trained personnel followed strict cleaning protocols and used personal protective equipment to ensure thorough and safe maintenance, supporting both animal welfare and the reliability of research outcomes.

Mating procedures

During estrus, female mice were mated with males at a 1:4 ratio, with mating occurring at 4 PM. After overnight mating, females were examined for sperm plugs and underwent vaginal smears to confirm pregnancy, with the detection of spermatozoa marking day one of gestation. The presence of a yellowish vaginal plug, a mixture of female and male secretions, further confirmed pregnancy [26,27]. Pregnant mice were separated, while non-pregnant mice were re-mated.

Sterilization and administration of probiotic-rich fermented milk

The control group received sterilized milk (10 mL/kg BW), while the intervention group received fermented milk containing *L. plantarum* IIA-1A5 (10^7 CFU/mL). To ensure that the sterilized milk was free of microbial contamination, pasteurization was employed, heating the milk to a specific temperature to eliminate bacteria and other microorganisms without compromising its nutritional value. The sterilized milk was administered orally to the mice using an 18–20-gauge bulb-tipped feeding tube, designed to deliver liquid directly into the stomach without causing harm [28]. Administration occurred once daily during the organogenesis period of pregnancy, from days 6 to 15 of pregnancy, which is critical for the development of fetal organs and structures. To ensure unbiased results and maintain study integrity, a single-blind method was employed, in which the personnel administering the milk were unaware of the group assignments of the mice, thus preventing potential bias in the administration process.

Surgical procedures

On day 18 of pregnancy, a laparotomy—an aseptic surgical incision into the abdominal cavity—was performed. The incision for the teratogenic test in mice typically follows a midline or slightly lateral approach along the lower abdomen, measuring approximately 1.5 to 2.5 centimeters, depending on the size of the mouse and the number of fetuses to be accessed. This procedure was executed by a pharmacologist from the Laboratory of Animal Research, Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia, employing a single-blind method.

After the procedure, the mice were humanely sacrificed via decapitation to facilitate examination of the uterine contents and evaluation of fetal morphology and skeletal development. The surgical approach involved sequentially incising the abdominal muscle layers to access and extract the fetuses, which were then separated from the placenta using surgical blades or a scalpel [29].

Maternal body weight assessment

Maternal body weight measurements were taken at two time points, starting with an initial pre-gestation measurement to establish a baseline before mating. Gestational measurements were recorded on gestational day (GD) 0 and 18. Incremental measurements were used to monitor trends in maternal weight gain, with total weight gain calculated by comparing the final gestational weight to the initial weight. The measurement used a digital balance scale with 0.01-

gram accuracy. To reduce bias, the measurement was conducted by trained personnel from the Laboratory of Animal Research who were blinded to the experimental groups.

Fetal assessment

Following laparotomy, fetal weight and the number of developing fetuses in each group were assessed. Fetal body weight measurements were taken immediately after birth to accurately assess fetal development. The number of fetuses was the total count of embryos or fetuses present in a pregnant animal, including deceased fetuses. Each fetus was cleaned with filter paper to remove residual fluids and blood before weighing, and all measurements were recorded.

The assessment of fetal morphology involved a thorough examination of external features and skeletal evaluation. One fetus from each parent was used for external examination, and one was selected for skeletal examination. This resulted in a total of six fetuses for external examination and six fetuses for skeletal examination. For external examination, the fetuses were fixed in Bouin's solution (75% aqueous saturated picric acid, 25% formalin, and 5% glacial acetic acid) for 14 days, achieving a firm and yellowish consistency [29]. An external examination was conducted on the ears, eyes, tail, and palate to identify any abnormalities. Markers for different body parts were visually inspected: the head was assessed for shape and size, eyelids were checked for closure, and ears were evaluated for positioning and development. Each feature was examined carefully to detect any potential deformities.

Meanwhile, for skeletal evaluation, the fetus from each mother was immersed in alizarin red solution for three days, rendering the tissues transparent and highlighting ossified skeletal structures. After this process, assessments included evaluations of bone structure, morphology, quantity, size, positioning, and staining intensity. Specific markers, such as the frontal bones and parietal bones, were identified based on their characteristic shapes and locations in the skull.

Study outcomes

Outcome measures comprised maternal weight fluctuations, weight and number of the fetuses, and their morphology and skeletal structure. Scoring was quantified through a comprehensive examination of fetal bone structure and morphology, measuring and recording parameters such as bone quantity, size, positioning, and staining intensity using established scoring systems or grading scales. A score of one was given for the presence of the assessed morphology or feature, while zero was given for absence. This evaluation process was conducted by pharmacologists to ensure accurate analysis and interpretation of findings. All results were systematically documented and compared against control groups to identify any significant differences or anomalies [29,30].

Statistical analysis

Continuous data were presented as mean and standard deviation (SD) for normally distributed data and as median (minimum-maximum) for non-normally distributed data; categorical data were presented as frequency and percentages. The Shapiro-Wilk test was utilized to assess data normality. For normally distributed data, an independent Student t-test was conducted [31], while the Mann-Whitney test was used for non-normally distributed data [32] to compare between the groups. The SPSS version 24.0 software (IBM SPSS, Chicago, Illinois, USA) was employed for data analysis, with $p \leq 0.05$ considered statistically significant.

Results

Effect of *L. plantarum* IIA-IA5 fermented milk on maternal and fetal body weight and fetal numbers

Initially, maternal body weight in the intervention group was significantly higher (24.98±3.30 g) than in the control group (23.55±1.30 g; $p<0.001$). However, during gestation, the control group exhibited a greater mean body weight (40.88±4.40 g) compared to the intervention group (39.72±4.32 g; $p<0.001$). The weight gain was significantly higher in the control group (17.33±3.99 g) than in the intervention group (14.73±1.52 g; $p<0.001$). For fetal body weight, the control group had a mean weight of 1.10±0.20 g, significantly greater than the intervention group's mean of 0.73±0.57 g ($p=0.027$). Additionally, the control group had a higher mean

number of fetuses (8.83±3.87) compared to the intervention group (6.83±5.46; p=0.028). These findings indicate a significant impact of the intervention on maternal and fetal parameters (**Table 1**).

Table 1. Effect of *Lactiplantibacillus plantarum* IIA-IA5 fermented milk on maternal and fetal body weight and fetal numbers

Variables	Groups		*p*-value [a]
	Control, mean±SD	Intervention, mean±SD	
Maternal body weight			
Initial (g)	23.55±1.30	24.98±3.30	<0.001
Gestational (g)	40.88±4.40	39.72±4.32	<0.001
Incremental (g)	17.33±3.99	14.73±1.52	<0.001
Fetal body weight (g)	1.10±0.20	0.73±0.57	0.027
Number of fetuses	8.83±3.87	6.83±5.46	0.028

[a]Analyzed using Student t-test

Effect of *L. plantarum* IIA-IA5 fermented milk on fetal external morphological features

In the present study, each mother mouse typically carried approximately ten fetuses and was allocated into two groups: one group was subjected to Bouin's staining for morphological assessment (**Figure 1**), while another group was subjected to alizarin red staining for skeletal analysis (**Figure 2**). The morphological assessment of mouse fetuses stained with Bouin's solution revealed no significant differences between the control and intervention groups (**Figure 1** and **Table 2**). Both groups demonstrated uniform development across all parameters, including head, eyelid, ears, cleft palate, foot, hand, tail, and skin, with a mean score of 1.00±0.00 for each feature.

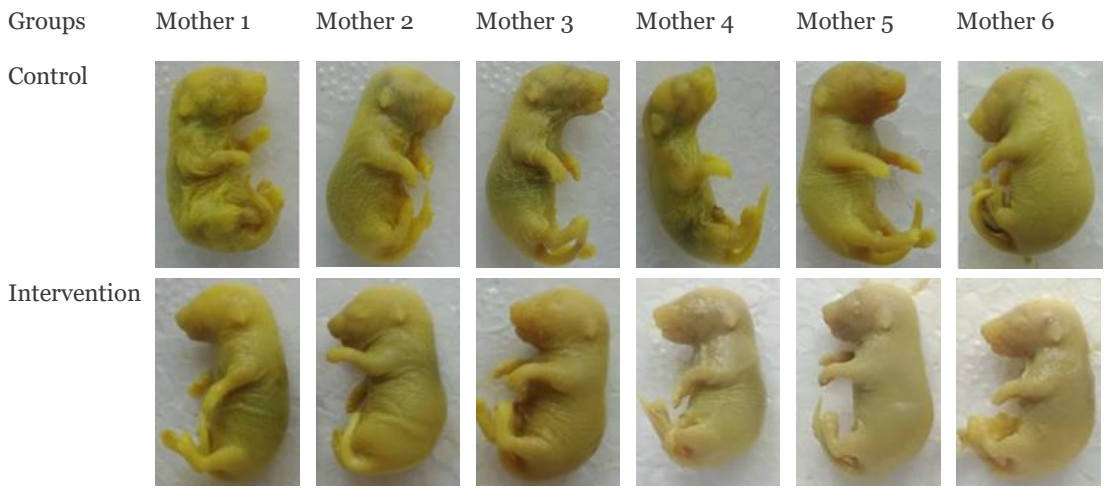

Figure 1. Representative fetal morphology from each mother mouse from both intervention and control groups.

No resorption sites, growth retardation, or hemorrhage were observed, with all measurements recording 0.00±0.00. These findings indicate that fetuses in both groups exhibited normal morphology without detectable abnormalities (**Table 2**).

Table 2. Evaluation of morphological features in mouse fetuses stained with Bouin's solution; one fetus from one mother was examined

Morphology	Control group, n							Intervention group, n						
	C1	C2	C3	C4	C5	C6	Mean±SD	I1	I2	I3	I4	I5	I6	Mean±SD
Head	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00
Eyelid	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00
Ears	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00
Cleft palate	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00
Foot	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00
Hand	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00

Morphology	Control group, n							Intervention group, n						
	C1	C2	C3	C4	C5	C6	Mean±SD	I1	I2	I3	I4	I5	I6	Mean±SD
Tail	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00
Skin	1	1	1	1	1	1	1.00±0.00	1	1	1	1	1	1	1.00±0.00
Resorpsion site	0	0	0	0	0	0	0.00±0.00	0	0	0	0	0	0	0.00±0.00
Growth retardation	0	0	0	0	0	0	0.00±0.00	0	0	0	0	0	0	0.00±0.00
Hemorrhage	0	0	0	0	0	0	0.00±0.00	0	0	0	0	0	0	0.00±0.00

A value of 1 represents the morphology that appears and a value of 0 represents the occurrence of formations that do not appear. C1-C6: mother 1 to 6 in the control group; I1-I6: mother 1 to 6 in the intervention group; One fetus represented one mother

Effect of *L. plantarum* IIA-IA5 fermented milk on bone development

The skeletal development of mouse fetuses stained with alizarin red solution showed no visible differences between the control and intervention groups for bone parameters (**Figure 2**). Both groups had the same average number of frontal, parietal, intraparietal, exoccipital, supraoccipital, nasal, pre-maxilla, mandibular, thoracal, lumbar, sternum, ribs, ulna, radius, humerus, femur, tibia, fibula, metatarsal, metacarpal, and scapula bones.

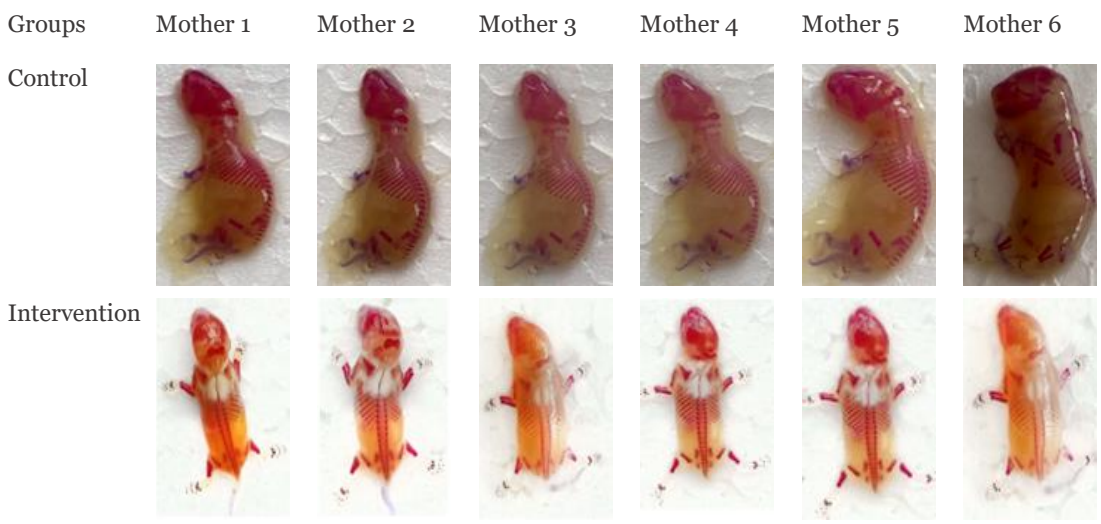

Figure 2. Representative of fetal bone development from each mother mouse from both intervention and control groups.

Discussion

At the beginning of the study, right after acclimatization, the control and intervention groups showed a significant difference in body weight. This is an interesting finding since the test was conducted under controlled conditions. However, the tested animals could still be used as they did not experience weight loss during the acclimatization period. This difference was likely attributed to variations in basal metabolic rates among individual animals, as explained elsewhere [33].

In 14-day-old mouse embryos, assessments of the circulatory, musculoskeletal, nervous, and craniofacial systems revealed no developmental delays among the groups in the present study. Fermented milk products are often enriched with bioactive compounds that may enhance the bioavailability of essential nutrients for fetal development. These compounds can influence the metabolism of nutrients crucial for bone formation, further supporting healthy ossification [34]. Visual examinations of external features indicated no physical abnormalities or treatment-related anomalies in the head, nose, mouth, or internal organs. Consistent with previous studies, these findings suggest that fermented milk containing *L. plantarum* IIA-IA5 is non-toxic and safe for consumption [20,35]. It may have potential applications in treating iron deficiency during pregnancy and as an anti-cancer agent [15,22,36,37]. Thus, it can be concluded that *L. plantarum* IIA-IA5 exhibits no teratogenic effects on soft tissues in mouse fetuses at the tested doses.

Significant skeletal ossification occurs in mice during the late fetal period, which is crucial for developing a mature skeletal system [38]. Evaluating bone ossification is essential in teratogenic studies to ensure safe fetal development [26,39,40]. In the present study, the

ossification of 14-day-old mouse fetuses was examined, revealing no significant differences in ossification centers of the skull, vertebrae, hyoid, forelimbs, or hindlimbs between the treatment and control groups. The treatment did not affect ossification centers in the sternum, caudal vertebrae, metatarsals, metacarpals, or phalanges. Importantly, no adverse effects on fetal maturation were observed. According to recent studies, the probiotic *L. plantarum* IIA-1A5 may enhance gut health and nutrient absorption without negatively impacting skeletal development [41,42]. Probiotics are known to modulate gut microbiota, leading to improved digestion and absorption of essential nutrients like calcium and vitamin D, which are critical for bone development. This may promote healthy fetal ossification by supporting maternal nutrition without inducing adverse effects [41].

This study has notable strengths, including its focus on the potential benefits of probiotics for maternal and fetal health and its experimental design, which features a control group that allows for robust comparisons. The detailed characterization of the *L. plantarum* IIA-1A5 strain, dosage, and administration method enhances reproducibility. However, limitations include the use of an animal model, which may not directly translate to human outcomes, and a small sample size of six pregnant mice per group, which may limit generalizability. Additionally, the study primarily examines body weight, morphology, and skeletal development, potentially overlooking other maternal and fetal health aspects.

Further research is needed to confirm these findings in humans and to investigate the effects of *L. plantarum* IIA-IA5 on embryonic development. Future studies should focus on its biological mechanisms, including impacts on gut microbiota, nutrient absorption, immune responses, and systemic factors. Determining the optimal probiotic dose for healthy development and assessing long-term effects on progeny into adulthood is crucial. Additionally, comparing different probiotic strains will help ascertain whether the observed effects are specific to *L. plantarum* IIA-IA5. Exploring interactions with other dietary components and analyzing changes in gut microbiota composition can further clarify the link between microbial health and embryonic development. Ultimately, clinical trials in humans are necessary to assess safety and efficacy.

Conclusion

The findings indicate that fermented milk enriched with *L. plantarum* IIA-1A5 is safe for consumption, as no morphological or skeletal abnormalities were observed in fetuses. This suggests that the probiotic strain does not adversely affect fetal development, highlighting its potential as a promising dietary intervention for pregnancy health. However, further research involving larger sample sizes and human subjects is needed to validate these results and assess the long-term health effects on mothers and offspring.

Ethics approval

The protocol of the present study was reviewed and approved by the Ethical Committee of Research, Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia (Approval number 55/UN.16.10.D.KEPK-FF/2023).

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Competing interests

All authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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References

1. Fitria N, Sari YO, Putry AR, *et al.* Future challenge on probiotics uses from fermented milk on the endocrine disorder in human. IOP Conf Ser Earth Environ Sci 2021;888(1):1-7.
2. Melia S, Juliyarsi I, Kurnia YF, *et al.* Effect of fermented milk *Pediococcus acidilactici* BK01 on cholesterol and microbiota in Wistar mice intestine. J Adv Vet Anim Res 2023;10(1):64-71.
3. Monteagudo-Mera A, Rastall RA, Gibson GR, *et al.* Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Appl Microbiol Biotechnol 2019;103(16):6463-6472.
4. Mazziotta C, Tognon M, Martini F, *et al.* Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells 2023;12(1):184.
5. Sharifi-Rad J, Rodrigues CF, Stojanović-Radić Z, *et al.* Probiotics: Versatile bioactive components in promoting human health. Med Lith 2020;56(9):1-30.
6. Darbandi A, Asadi A, Mahdizade Ari M, *et al.* Bacteriocins: Properties and potential use as antimicrobials. J Clin Lab Anal 2022;36(1):1-40.
7. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, *et al.* Mechanisms of action of probiotics. Adv Nutr Bethesda Md 2019;10 suppl 1:S49-S66.
8. Fatdillah H, Desriani, Melia S, *et al.* Variant alpha and beta biodiversity of the genus in dadiah through deep sequencing 16S Ribosomonal RNA genes. IOP Conf Ser Earth Environ Sci 2021;888(1):12-17.
9. Desriani, Azamris, Ghaissani SS, *et al.* Design and characterization of a SYBR Green I-based melting curve method for investigation of HER2I655V polymorphism in breast cancer. J Genet Eng Biotechnol 2021;19(1):6.
10. Kim NN, Kim WJ, Kang SS. Anti-biofilm effect of crude bacteriocin derived from *Lactobacillus brevis* DF01 on *Escherichia coli* and *Salmonella Typhimurium*. Food Control 2019;98:274-280.
11. Arief II, Budiman C, Jenie BSL, *et al.* Plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 displays bactericidal activity against *Staphylococcus aureus*. Benef Microbes 2015;6(4):603-613.
12. Arief II, Jakaria, Suryati T, *et al.* Isolation and characterization of plantaricin produced by Lactobacillus plantarum strains (IIA-1A5, IIA-1B1, IIA-2B2). Media Peternak 2013;36(2):91-100.
13. Diep DB, Straume D, Kjos M, *et al.* An overview of the mosaic bacteriocin pln loci from *Lactobacillus plantarum*. Peptides 2009;30(8):1562-1574.
14. Ningtiyas WD, Arief II, Budiman C, *et al.* Inhibition of human cervical cancer HeLa cell line by meat-derived lactic acid bacteria of *Lactobacillus plantarum* IIA-1A5 and *Lactobacillus acidophilus* IIA-2B4. Pak J Biol Sci 2021;24(12):1340-1349.
15. Adiyoga R, Arief II, Budiman C, *et al.* In vitro anticancer potentials of *Lactobacillus plantarum* IIA-1A5 and *Lactobacillus acidophilus* IIA-2B4 extracts against WiDr human colon cancer cell line. Food Sci Technol Braz 2022;42(1):1-7.
16. Kia KW, Arief II, Sumantri C, *et al.* Plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 retards pathogenic bacteria in beef meatball stored at room temperature. Am J Food Technol 2016;11(1-2):37-43.
17. Fitria N, Lailaturrahmi L, Sukma A. The effect of consuming fermented milk containing probiotics during pregnancy. IOP Conf Ser Earth Environ Sci 2024;1341(1):1-7.
18. Syahriandra S, Rivai MI, Irwan I, *et al.* The effect of giving fermented milk starter lactococcus lactis D4 dadih on obstructive jaundice: An in vivo study. Biosci Med J Biomed Transl Res 2022;6(15):2761-2766.
19. Melia S, Juliyarsi I, Kurnia YF, *et al.* Profile of stingless bee honey and microbiota produced in West Sumatra, Indonesia, by several species (Apidae, Meliponinae). Vet World 2024;17(4):785-795.

20. Yi L, Min JT, Jun CL, *et al.* Buffalo yogurt fermented with commercial starter and *Lactobacillus plantarum* originating from breast milk and its lowering blood pressure in pregnant hypertensive rats. J Dairy Sci 2024;107(1):62-73.
21. Bouhafs L, Moudilou EN, Exbrayat JM, *et al.* Protective effects of probiotic *Lactobacillus plantarum* BJ0021 on liver and kidney oxidative stress and apoptosis induced by endosulfan in pregnant rats. Ren Fail 2015;37(8):1-9.
22. OjiNjideka HN, Pezley L, Steffen A, *et al.* Feasibility study of *Lactobacillus Plantarum* 299v probiotic supplementation in an urban academic facility among diverse pregnant individuals. Nutrients 2023;15(4):1-17.
23. BPOM RI. Guidelines for in vivo preclinical toxicity testing. Food and drug supervisory agency regulation, number 10 of 2022. Jakarta: BPOM RI; 2022.
24. Bolarinwa OA. Sample size estimation for health and social science researchers: The principles and considerations for different study designs. Niger Postgrad Med J 2020;27(2):67-75.
25. Percie du Sert N, Hurst V, Ahluwalia A, *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. BMC Vet Res 2020;16(1):242.
26. Byers SL, Wiles M V, Dunn SL, *et al.* Mouse estrous cycle identification tool and images. PloS One 2012;7(4):e35538.
27. Gonzalez G. Determining the stage of the estrous cycle in female mice by vaginal smear. Cold Spring Harb Protoc 2016;2016(8):10.1101/pdb.prot094474.
28. Institutional Animal Care and Use Committee (IACUC), University of California San Francisco. Routes and volumes of administration in mice - IACUC Guideline 2021. California: University of California San Francisco; 2021.
29. Abebe M, Asres K, Bekuretsion Y, *et al.* Teratogenic effect of high dose of *Syzygium guineense* (Myrtaceae) leaves on wistar albino rat embryos and fetuses. Evid Based Complement Alternat Med 2021;2021:6677395.
30. Isoherranen N, Spiegelstein O, Bialer M, *et al.* Developmental outcome of levetiracetam, its major metabolite in humans, 2-pyrrolidinone N-butyric acid, and its enantiomer (R)-alpha-ethyl-oxo-pyrrolidine acetamide in a mouse model of teratogenicity. Epilepsia 2003;44(10):1280-1288.
31. Fivi MD, Najmiatul F. Association of omega 3 and omega 6 and child development: A systematic review. Nutr Clin Diet Hosp 2022;42(2):98-104.
32. Field A. Discover statistics using SPSS-third edition. London: SAGE Publications Ltd; 2011.
33. Pettersen AK, Marshall DJ, White CR. Understanding variation in metabolic rate. J Exp Biol 2018;221(Pt 1):jeb166876.
34. Sarkar A, Khan AS, Kaul R, *et al.* The link between gut microbiome, nutraceuticals, diet, and diabetes mellitus: A bibliometric and visual analysis of research and emerging trends from 2012 to 2022. Discov Public Health 2024;21:107.
35. Vonderheid SC, Tussing-Humphreys L, Park C, *et al.* A Systematic review and meta-analysis on the effects of probiotic species on iron absorption and iron status. Nutrients 2019;11(12):2938.
36. Fåk F, Ahrné S, Molin G, *et al.* Maternal consumption of *Lactobacillus plantarum* 299v affects gastrointestinal growth and function in the suckling rat. Br J Nutr 2008;100(2):332-338.
37. Susmiati S, Fitria N, Khairina I, *et al.* Effect of fermented milk Lactiplantibacillus pentosus HBUAS53657 on blood glucose, lipid profiles and inflammation in high-fat diet-induced mice. Romanian J Diabetes Nutr Metab Dis 2023;30(4):440-446.
38. Lovsin BE, Zabavnik PJ. Development of the skeleton in mouse fetuses of both sexes between 14.5 and 18.5 days post coitum. Pol J Vet Sci 2008;11(2):147-149.
39. Khaksary MM, Najafzadeh VH, Bakhtiari E. The effects of cyclophosphamide, melatonin and carvedilol on neural tube and skeletal system of mice fetuses in prenatal period. Ann Anat 2011;193(5):459-465.
40. Khaksary MM, Ranjbar R, Arzi A, *et al.* A comparison study of effects of Echinacea extract and levamisole on phenytoin-induced cleft palate in mice. Regul Toxicol Pharmacol 2006;46(3):163-166.
41. Rocchetti MT, Russo P, Capozzi V, *et al.* Bioprospecting antimicrobials from *Lactiplantibacillus plantarum*: Key factors underlying its probiotic action. Int J Mol Sci 2021;22(21):12076.
42. Weaver CM, Alexander DD, Boushey CJ, *et al.* Calcium plus vitamin D supplementation and risk of fractures: An updated meta-analysis from the National Osteoporosis Foundation. Osteoporos Int 2016;27(1):367-376.