



# Micronutrient Content and Total Lactic Acid Bacteria of Dadiah Pudding as Food Supplementation for Pregnant Women

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## Abstract

**BACKGROUND:** Dadiah, traditional yogurt from Indonesia, which is known as a source of probiotics, also contains micronutrients.

**AIM:** This descriptive study aimed to determine whether additional ingredients and processes during the manufacture of Dadiah pudding maintain the iron, zinc, calcium, and total lactic acid bacteria (LAB) contents.

**METHODS:** Dadiah pudding was made using Dadiah originated from Bukittinggi, West Sumatra. Micronutrient levels were analyzed according to Indonesian National Standard 01-2896-1998. The total LAB were counted by inoculating samples on Man, Rogosa, and Sharpe agar with serial dilution, and morphological identification was carried out using gram stain.

**RESULTS:** In 100 g of original Dadiah, mango and chocolate Dadiah pudding contained 347.98, 276.61, and 279.29 mg of calcium; 4.87, 3.75, and 6.31 mg of zinc; 6.53, 6.60, and 9.39 mg of iron; and  $6.4 \times 10^9$ ,  $6.1 \times 10^9$ , and  $2.4 \times 10^9$  CFU/ml LAB, respectively.

**CONCLUSION:** This study found that modifying the original Dadiah into Dadiah pudding has been proved to affect the concentration of calcium, zinc, and iron and to maintain total LAB. We suggest that consuming Dadiah pudding may be a good choice as a food supplementation for pregnant women to optimize the golden period outcomes.

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## Introduction

The first 1000 days of a child's life are a golden period for child growth and development. Maternal nutritional adequacy is positively associated with birth outcomes. In general, poor nutrition during pregnancy caused micronutrient deficiency [1]. Low intake of calories and some micronutrients such as iron and zinc during pregnancy can result in low birth weight [2], [3], [4]), the major determinant for stunted growth [5]. Iron and calcium deficiency in pregnant women also increased the risk of preterm birth, which led to early neonatal and infant mortality [2], [6]. Many women go through their entire pregnancy without reaching the minimum required intake of these micronutrients [7], [8], [9]. Undernutrition of a child in the first 2 years also correlated with the immaturity of gut microbiota [10], [11]. Up to 20% of stunting was thought to occur in the uterus. New evidence has found that there are host-microbial interactions in the uterus and that the fetus can inherit maternal microbes before birth [12], [13]. Probiotic administration during pregnancy

affected the gut microbiota of the mother [14]. Dietary intake makes up more than 20% of gut microbiota variations in humans [15] and micronutrients act as an ecological modulator of intestinal microbes [16].

Dadiah or "Dadih," a yogurt-like product that fermented from West Sumatran swamp buffalo's milk is used as a bottom-up approach to tackling the undernutrition problem. Although the manufacturing of Dadiah is still in a traditional process and has not met any national standards [17] or international standards [18] for yogurt and fermented milk, Dadiah provides safety and health for people who consume it. There are no pathogenic bacteria in Dadiah [19]. Several studies had demonstrated that Dadiah had beneficial effects on the intestinal health of mothers and children due to its nutritional value and its role as a probiotic [19], [20], [21], [22], [23], [24]. Dadiah had more calories than yogurt from cow's milk [19]. Dadiah also contains micronutrients such as calcium, iodine, zinc [25], and several B vitamins and riboflavin, niacin, and folic acid [26]. Different

sources of Dadiah had different amounts of macro- and micronutrients content [19], [27]. Different regions of buffalo's milk source, as a probiotic product, also caused different numbers of viable lactic acid bacteria (LAB) [29], [20], [28]. LAB produced various kinds of enzymes that play a role in host metabolism and make Dadiah easier to digest than raw fresh buffalo milk [29].

Dadiah is typically consumed by West Sumatran people as one of ethnic foods of the Minangkabau culture. It was served originally with rice by adding sliced shallot and red chilies, mixed with sticky rice and palm sugar as "ketan dadiah" or with traditional glutinous rice flakes called "ampiang dadiah." The shelf life of the original Dadiah was only 3 days at room temperature with optimum growth of LAB and nutrient value; it was 48 h at 20°C–45°C [29]. Dadiah stored at 8°C temperature was longer in duration, 6–12 days. Lower temperature can slow down the fermentation process and thus prevent the metabolic activity of pathogen bacteria and inhibit fungal growth [30]. Sensory test results indicated that different regions resulted in differences in color, taste, texture, and general acceptance of Dadiah. Panelists preferred Dadiah that originated from Tanah Datar with a slightly white color, less acidic taste, and a soft texture like that of a pudding than Dadiah from Agam [19]. Therefore, Dadiah is now also made into Dadiah pudding, which is served as a cold snack for pregnant women in West Sumatra [31]. Storing the Dadiah pudding at a lower temperature made the shelf life longer than that of the original Dadiah [32]. Dadiah pudding may also be more tolerant for consumption due to its lower acidity and aroma than those of original Dadiah. However, it is not yet known whether the micronutrient content and total LAB in the Dadiah pudding and the addition of ingredients and the manufacturing process during making of Dadiah pudding will affect the micronutrient content and LAB growth in Dadiah. This research also modified the ingredients and processes in manufacturing Dadiah pudding in Helmizar's previous study [31] to get more optimal results. Moreover, data on micronutrient intake for pregnant women in West Sumatra is still limited. Compared to the other studies, this is the first study that investigates the micronutrient contents in Dadiah pudding. This study aims to determine the micronutrient content and total LAB in Dadiah and Dadiah pudding from Bukittinggi, West Sumatra in relation to fulfill micronutrient intake of pregnant women in the province by giving local food.

## Materials and Methods

### Materials

Dadiah samples (Figure 1) were taken from a village of Gaduik region, Bukittinggi, West Sumatra. Dadiah was made in the traditional way without being

boiled, pasteurized, or inoculated with a starter culture. Fresh unheated buffalo milk was poured into a bamboo tube and then covered with banana leaves or plastics. It was incubated at room temperature (28°C–30°C) for 48 h to allow natural fermentation. Original Dadiah has a cream-white color, smooth consistency, shiny surface, delicious aroma, and sour taste [29].

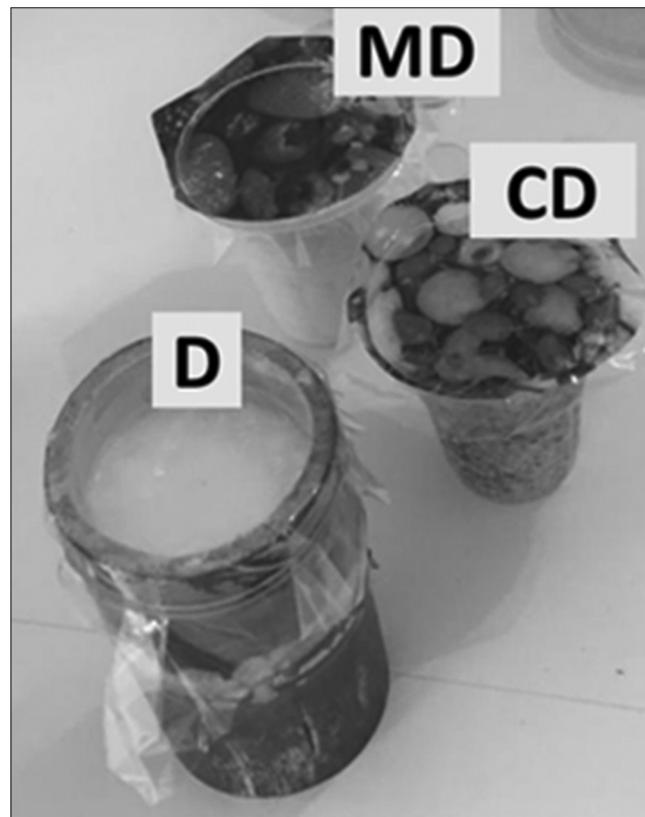


Figure 1: D (Dadiah), MD (Mango Dadiah), and CD (Chocolate Dadiah)

Dadiah pudding was made by modifying a recipe from Helmizar [31]. There were two flavors of Dadiah pudding: Mango and chocolate (Figure 1). The ingredients used in this study are 75 g of 48-h fermented buffalo's milk, 15 g of pudding powder (Nutrijel pudding of mango milk or chocolate produced by PT Forisa Nusapersada with BPOM RI MD 663310235304), and 50 ml of water. By contrast, previous research used 75 g of 24-h fermented buffalo's milk mixed with 5 g Dadiah as a starter, 15 g of pudding powder, and 50 ml of water [31]. Thus, there was no starter used in making Dadiah pudding in this study. Pudding powder was mixed with the water, and the mixture was cooked and stirred until it boiled. Pudding was cooled at room temperature to  $\pm 60^{\circ}\text{C}$ – $70^{\circ}\text{C}$  and then mixed well with Dadiah. Dadiah pudding was poured into molds, chilled in a refrigerator for it to be ready to be served.

### Maternal intake of micronutrients

This research was part of a randomized, double-blind, and controlled trial, "The Effect of Dadiah on

Pregnant Women and Birth Outcome in West Sumatra,” conducted with 208 pregnant women in Agam, Lintau, and Padang Panjang, West Sumatra, Indonesia during 2018–2020. The subjects were healthy pregnant women aged 17–44 years who were in the first two trimesters of pregnancy and had registered for antenatal care examination at the selected Primary Health Centers in 2018–2020. They gave their consent to participate in this study. Pregnant women with a previous diagnosis of HIV/AIDS, TBC, hepatitis B, multiple pregnancies, high-risk pregnancies (hypertension, preeclampsia, diabetes, and history of bleeding) were excluded from the study. Maternal intake of calcium, zinc, and iron during the first trimester was assessed using the validated Minang semi-quantitative food frequency questionnaire developed by Lipoeto [33]. Data were managed and analyzed descriptively using IBM SPSS Statistics for Windows (SPSS) version 20.0 (IBM, Armonk, New York) and presented as tables.

### Micronutrients analysis

The calcium, zinc, and iron levels were tested at Industrial Research and Standardization Center (Baristand) Laboratory, Padang, West Sumatra according to Indonesian National Standard (SNI) 01-2896-1998.

### Macroscopic and total LAB count

De Man, Rogosa, and Sharpe (MRS) Agar (Oxoid, Thermo Fisher Scientific, Hampshire, UK) media, distilled water, and required materials were prepared. The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Universitas Andalas. For an initial  $10^{-1}$  dilution, a total of 100  $\mu\text{l}$  of each sample was dissolved in 900  $\mu\text{l}$  distilled water in a test tube, the solution was vortexed until it was homogeneous. This procedure was repeated until the  $10^{-6}$  dilution was attained. From the appropriate dilution ( $10^{-5}$ – $10^{-6}$ ), a 100  $\mu\text{l}$  sample was taken and inoculated on a Petri dish containing MRS agar media and then flattened with a sterilized hockey stick. The inoculum was placed into the anaerobic jar and incubated for 48 h at 37°C. There were three Petri dishes marked as codes D for Dadijah, CD for chocolate Dadijah pudding, and MD for mango Dadijah pudding. After 48 h, the growing colonies of LAB were observed as white-beige smooth round. The number of LAB colonies was expressed as colony-forming units per milliliter of sample and calculated using the formula (1).

$$\text{CFU/ml} = \text{number of colonies} \times 1/\text{dilution} \times 1/\text{sample weight} \quad (1)$$

### Morphological identification

Bacterial culture colonies were taken and placed on a microscopic slide. The crystal violet dye

was applied to a heat-resistant smear and left for 1 min. Then, it was rinsed with distilled water and dried with air, followed by dropping of iodine, allowed to react for 1–2 min, and rapid decolorization with ethanol. Finally, X was counterstained with safranin for 1 min and a rinse with distilled water and air-dried. The results were observed under a microscope at 1000 $\times$  magnification.

## Results and Discussion

### Maternal intake of micronutrients

The subjects comprised 208 healthy pregnant women whose mean values of age and gestational age were  $29.28 \pm 5.42$  years and  $13.60 \pm 4.14$  weeks, respectively. Our study revealed that more than half of pregnant women had inadequate intakes of calcium (89.9%), zinc (69.7%), and iron (74.0%). The inadequacy of dietary intake of these micronutrients as compared to the Indonesian Recommended Dietary Allowances (RDAs) is indicated in Table 1.

**Table 1: Daily micronutrient intake of subjects**

Variable	Mean (SD)	Median	Min.–Max.	RDA	% RDA
Calcium (mg)	562.70 (355.35)	457.43	109.60–2328.59	1,300	43.3
Zinc (mg)	8.22 (3.59)	7.39	2.42–21.78	12	68.5
Iron (mg)	15.99 (5.80)	15.44	4.81–31.62	26	61.5

SD: Standard deviation; RDA: Recommended dietary allowance for Indonesian pregnant women aged 16–49 years.

The daily mean and percentage of RDAs consumed for calcium intake in this study were lower than those in a previous study on 203 Minangkabau pregnant women above 28 weeks of pregnancy in two coastal districts (Pariaman and Pasaman Barat) and two mountainous areas (Solok and Tanah Datar) [7]. However, these results were higher than those of previous research by Bardosono [8] on maternal micronutrient deficiency during the first trimester among 143 pregnant women living in Jakarta. This may be caused by nausea as the most (70.6%) reported complaints found among the participants related to early pregnancy [8]. Based on this evidence, pregnant women went through the first trimester without attaining the minimum required intake of these micronutrients that need further intervention to plan a healthy pregnancy outcome.

### Micronutrient analysis

According to the Indonesian RDA, pregnant mothers aged 16–49 years need approximately 1300 mg of calcium, 12 mg of zinc, and 26 mg of iron daily [34]. Table 2 presents concentrations of calcium, zinc, and iron in Dadijah and Dadijah pudding obtained in this study. There were 347.98 mg of calcium (Ca), 4.87 mg of zinc (Zn), and 6.53 mg of iron (Fe) in 100 mg of Dadijah (D) from Gaduik, Bukittinggi. Consumption

of the original Dadijah in this study by the mother during pregnancy may cover approximately one-third of calcium, two-fifth of zinc, and one-fourth of iron intake per day. The calcium and zinc levels found in our research were higher than those in a previous study on Dadijah micronutrient analysis from Agam and Tanah Datar district, West Sumatra. Dadijah Agam contained 190.54 mg of calcium and 0.7 mg of zinc, whereas Dadijah Tanah Datar had 247.93 mg of calcium and 0.9 mg of zinc in the same amount. However, there were no data on iron concentration in the Agam and Tanah Datar dadijah [25]. The difference in micronutrient content between these studies was probably due to differences in sources of buffalo milk as the main ingredient in Dadijah production. The quality, quantity, and composition of nutrition given to the buffalo affected the chemical characteristics of its milk [35].

**Table 2: Micronutrient content and total LAB of Indonesian dadijah, mango dadijah pudding and chocolate Dadijah pudding**

Content	Dadijah (D)	Mango Dadijah pudding (MD)	Chocolate Dadijah pudding (CD)
Micronutrient			
Ca (mg/100 g)	347.98	276.61	279.29
Zn (mg/100 g)	4.87	3.75	6.31
Fe (mg/100 g)	6.53	6.60	9.39
Total LAB (CFU/ml)	$6.4 \times 10^9$	$6.1 \times 10^9$	$2.4 \times 10^9$

LAB: Lactic acid bacteria.

The original Dadijah (D) had different compositions of calcium, zinc, and iron levels as compared to those in mango Dadijah pudding (MD) and chocolate Dadijah pudding (CD), as presented in Table 2. These differences might have resulted from the ingredients and the process while making Dadijah pudding. Modifying the original Dadijah into Dadijah pudding reduced calcium level from 347.98 mg in Dadijah to 276.61 mg in MD and 279.29 mg in CD. Calcium concentration was known to be closely related to the acidification step [36]. The highest concentration of zinc (Zn) and iron (Fe) was found in CD as compared to the original Dadijah (D) and MD may be influenced by the chocolate ingredient contained in the pudding powder. Chocolate is known as a source of zinc and provides some of iron [37]. Mango and CD may help meet around one-fifth of the daily calcium intake for pregnant women based on The Indonesian RDA. MD may cover a pregnant woman's daily requirement for one-third of zinc and one-fourth of iron intake, whereas CD may provide half of zinc and one-third of iron [34]. At present, the WHO recommends supplementation with 1500 mg/day of calcium, 15 mg/day of zinc, and 30 mg/day of iron [38], [39]. Thus, consumption of Dadijah pudding might help pregnant women through their pregnancy by achieving the minimum intake of these required micronutrients.

### Macroscopic and total LAB count

The original Dadijah samples used in this study had a white color, creamy taste, smooth texture, and milky aroma. According to Surono [29], good

quality of Dadijah has a cream-white color, smooth and shiny surface, uniform consistency, pleasant aroma, and sour taste and is free from cracks and air bubbles. Different Dadijah-producing areas resulted in significant differences in color, taste, texture, and general acceptance but not in the aroma. According to Helmizar [31], Dadijah originated from Tanah Datar had a slightly white in color and a less acidic taste and a soft texture compared to Dadijah Agam. This may be due to the quality of buffalo milk, which was affected by animal feed. The buffalos from Tanah Datar were fed with banto grass and Nepal stone or limestone that contains calcium carbonate ( $\text{CaCO}_3$ ) as food supplements. By contrast, Agam buffalos were fed with cogon grass (*Imperata cylindrica*) that contained several acidic substances such as chlorogenic acid, oxalic acid, malic acid, and citric acid. Moreover, the type of bamboo tube used to package the Dadijah also affected its macroscopic. Lapoh bamboo used as a container of Tanah Datar dadijah was smaller and thinner than Talang bamboo used to pack Agam dadijah. This bamboo was lower in porosity so that Tanah Datar dadijah's water content was higher than that of Agam and resulted in a soft texture [19].

As compared to the original Dadijah, the color of Dadijah pudding depends on the kind of the pudding powder used, peach color for MD, and light brown for CD. Dadijah pudding had a thinner consistency, and the milk aroma was not too strong because of the mango and chocolate flavor of pudding powder. Change in texture can be caused by the process of making Dadijah pudding by adding pudding powder and water to the original Dadijah.

Cultures were performed on MRSA, a selective medium that promotes the growth of lactic acid-producing bacteria. All samples indicated that the single colonies of original Dadijah (D), MD, and CD were white beige and had a smooth round shape, a convex surface, and a 1–2 mm diameter (Figure 2a). Our results are similar to the characteristics of isolates from Dadijah Lintau in studies by Amelia *et al.* [28] and

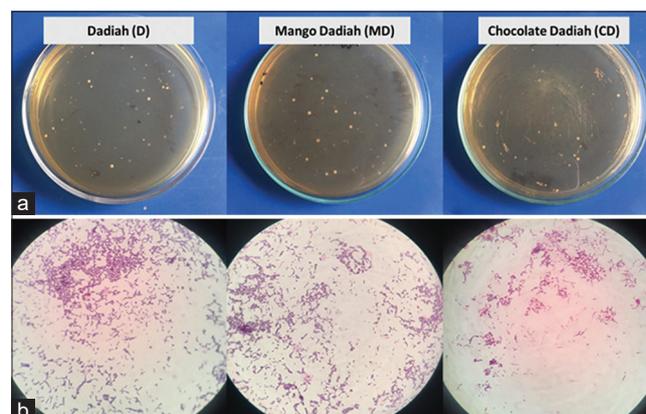


Figure 2: (a) Isolated colonies of lactic acid bacteria from samples cultured in de Man, Rogosa, and Sharpe Agar medium after incubation for 48 h at 37°C. (b) Gram stain results of samples showing Gram (+), non-motile rod and cocci bacteria

Purwati *et al.* [40] that found a round, edge slick, and convex shape; a white-beige color; and a 1.8 mm in diameter.

As a probiotic product, LAB in Dadiah has to be consumed alive and in an adequate amount to have a health benefit on the host [41]. The total colonies of LAB in Dadiah (D), MD, and CD are presented in Table 2. Our study found that total LAB in the original Dadiah ( $6.4 \times 10^9$  CFU/ml) was almost the same as those in MD ( $6.1 \times 10^9$  CFU/ml), whereas they were reduced ( $2.4 \times 10^9$  CFU/ml) in chocolate mango pudding. A decrease of 62.5% of total LAB in CD as compared to the original Dadiah may be due to the chocolate powder used as the additional ingredient in pudding powder. According to Peng *et al.* [42], the addition of cocoa into dairy products could improve the growth of *Lactobacillus*, common milk resident bacteria. However, these results indicated that modifying the original Dadiah into Dadiah pudding by adding various ingredients and certain manufacturing processes maintained the total viability of LAB. All samples were potential sources of probiotic food according to FAO/WHO because of concentrations were  $10^9$  CFU/ml, which met the minimum requirements of  $10^6$  CFU/ml [43].

LAB in Dadiah could be obtained mainly from raw fresh buffalo milk, bamboo tubes, or banana leaves [44]. The previous studies demonstrated that different sources of buffalo milk, animal feed, and various stages of the natural fermentation process resulted in different amounts of LAB in Dadiah;  $1.3\text{--}1.7 \times 10^8$  CFU/g in Dadiah from Bukittinggi [20],  $3.81 \times 10^7$  CFU/g in Dadiah from Lintau [45],  $1.9 \times 10^7$  CFU/g in Dadiah from Tanah Datar, and  $4.6 \times 10^6$  CFU/g in Dadiah from Agam [19]. Another study found that  $1.42 \times 10^8$  to  $3.80 \times 10^8$  CFU/g viable LAB in Dadiah originated from Bukittinggi and Padang Panjang [42]. The current result indicate that total LAB Dadiah air dingin was  $8.0 \times 10^8$  CFU/g [46], and  $7.1 \times 10^{10}$  CFU/ml total LAB Dadiah originated from Tanjung Bonai, Lintau Buo Utara, Tanah Datar District, West Sumatra [28]. A study reported the beneficial effects of probiotics to include improvement of intestinal health [47], metabolic efficiency [48], and the immune response [20], [23]. A bamboo tube used as a container for Dadiah also affected the growth of LAB. Talang bamboo used for Agam Dadiah was higher in porosity compared to Lapoh bamboo used for Tanah Datar Dadiah made total LAB of Agam Dadiah ( $4.6 \times 10^6$ ) was lower than Tanah Datar Dadiah ( $1.9 \times 10^7$ ) [19].

The growth of Dadiah bacteria was influenced by not only the animal feed, location, or method of fermentation but duration and temperature. According to Surono [29], our study used only 48-h fermented buffalo milk at  $20^\circ\text{C}\text{--}45^\circ\text{C}$  to get the optimum growth of LAB and its nutrient value [29]. Our research also indicated that reducing the pudding temperature to  $\pm 60^\circ\text{C}\text{--}70^\circ\text{C}$ , before mixing it with the original Dadiah

while making Dadiah pudding, helped maintain total LAB. This might be due to the thermotolerant capacity of some LAB to survive in thermal treatment, heating at  $70^\circ\text{C}$  in 60 min [49].

### Morphology identification

Colonies from all of the samples with the surrounding clear zone were randomly selected on each plate. From the Gram staining, LAB colonies were characterized by a non-motile rod and cocci Gram-positive bacteria (Figure 2b). Our results were similar to those of studies by Purwati *et al.* [40] and Amelia *et al.* [28], which indicated that the Gram-positive bacteria in Dadiah originated from Lintau, Tanah Datar Regency, West Sumatra. According to Amelia *et al.* [28], the LAB in Dadiah appeared as the rod-shaped Gram-positive bacteria. However, Purwati *et al.* [40] found that the LAB were cocci and bacilli. Surono [27] also found that all bacteria in Dadiah from Agam and Tanah Datar districts, West Sumatra were Gram-positive.

## Conclusion

Manufacturing the original Dadiah into Dadiah pudding with the addition of certain ingredients, and processes has been proved to change the concentration of calcium, zinc, and iron, and maintain the total LAB. There were 347.98, 276.61, and 279.29 mg of calcium; 4.87, 3.75 and 6.31 mg of zinc; and 6.53, 6.60, and 9.39 mg of iron in 100 g of original Dadiah and mango and CD. As a probiotic food, it also found  $6.4 \times 10^9$ ,  $6.1 \times 10^9$ , and  $2.4 \times 10^9$  CFU/ml total LAB in the original Dadiah and mango and CD. Therefore, Dadiah pudding can be a good choice as a food supplementation that is beneficial for prenatal supplementation and pregnancy outcomes. Consuming Dadiah pudding may facilitate attaining healthy pregnancy and optimal golden period outcomes. Dadiah pudding also has the potential to be a healthy food for daily consumption.

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