







## Greek-style yogurt enriched with shield aralia leaves improves lactation hormones in rats

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### ABSTRACT

#### Article History

Received: 12 September 2025

Revised: 7 November 2025

Accepted: 22 December 2025

Published: 15 January 2026

#### Keywords

Functional food

Hypogalactia

Lactation support

Oxytocin

Polyscias scutellaria yogurt

Prolactin.

Exclusive breastfeeding for six months is essential to provide adequate nutrition for infants. However, hypogalactia or insufficient breast milk production presents challenges during lactation. Herbal galactagogues have been traditionally used to maintain breast milk production. *Polyscias scutellaria* leaves are believed to possess galactagogue properties. This study aimed to evaluate the galactagogue properties of *P. scutellaria* yogurt in lactating rats. Twenty-five lactating rats were divided into five groups: unmated rats (UR), negative control lactating rats (NCR), positive control lactating rats (PCR), lactating rats treated with *P. scutellaria* yogurt at 2 g/kg BW (LRPSY1), and 4 g/kg BW (LRPSY2). Treatments were administered orally during a 3-week lactation period. The weekly body weight of dams and pups, prolactin, and oxytocin levels were examined. Prolactin and oxytocin were measured using enzyme-linked immunosorbent assay (ELISA). Results showed that the body weight of dams decreased while the body weight of pups increased following supplementation with *P. scutellaria* yogurt. Weekly prolactin and oxytocin levels increased significantly compared with the PCR group, which was treated with domperidone. At the third week of lactation, prolactin levels in the PCR group were 83.55 ng/mL, while LRPSY1 and LRPSY2 showed higher levels at 150.17 and 114.55 ng/mL, respectively. Furthermore, oxytocin levels at the third week of lactation were significantly higher in LRPSY1 (33.88 ng/mL) and LRPSY2 (35.33 ng/mL) compared with the PCR group (22.05 ng/mL). The increase in prolactin and oxytocin correlated with enhanced breast milk production. This study suggests that *P. scutellaria* yogurt has the potential to increase breast milk during lactation.

**Contribution/Originality:** This study contributes to the development of new functional foods utilizing dairy products for breastfeeding mothers. *Polyscias scutellaria* yogurt emerges as a novel galactagogue product. Herbal galactagogues incorporated into functional food products can enhance the benefits of galactagogue properties and nutritional value for breastfeeding mothers.

## 1. INTRODUCTION

Breastfeeding is widely recognized as the optimal source of infant nutrition, providing essential macronutrients, micronutrients, immunological components, and bioactive compounds that support growth and development during early life stages. The World Health Organization (WHO) recommends exclusive breastfeeding for the first six

months, with continued breastfeeding alongside appropriate complementary foods up to two years of age or beyond (Chęcińska-Maciejewska, Ciborek, Krauss, & Gibas-Dorna, 2024). Breast milk contains a rich array of nutrients, vitamins, and minerals necessary for optimal infant growth and development. It also includes vital nutrients and antibodies that contribute to better health outcomes for newborns, such as improved dietary habits, fewer hospitalizations, and enhanced cognitive and behavioral development (Agyekum, Codjoe, Dake, & Abu, 2022).

Nonetheless, mothers often face problems with infant feeding, including inadequate milk production and challenges in establishing breastfeeding practices, which may be exacerbated by physiological circumstances or insufficient lactogenesis (Feldman-Winter et al., 2020). Hypogalactia, often known as inadequate production of breast milk, affects approximately 10–15% of women who are breastfeeding. This condition is associated with early weaning, increased dependency on formula feeding, and a higher risk of infections and long-term metabolic abnormalities in infants. Contributing factors may include inadequate development of the mammary glands, hormonal changes, maternal stress, or inappropriate breastfeeding techniques (Bauer, Abele, & Graf, 2024). Galactagogues are medications that stimulate the mammary glands to produce more milk for nursing infants. Domperidone and metoclopramide are pharmacological interventions with galactagogue properties (Grzeskowiak, Wlodek, & Geddes, 2019). These agents function as dopamine antagonists, leading to elevated prolactin levels by inhibiting dopamine receptors in the hypothalamus. Although they facilitate prolactin secretion, their clinical use is limited due to potential adverse effects, including cardiac arrhythmias, fatigue, and gastrointestinal disturbances. This highlights the urgent need for alternative interventions that are safe, accessible, and effective in promoting lactation.

Herbal galactagogues have long been used in various cultures to enhance milk production (Zizzo, Amir, Moore, Grzeskowiak, & Rumbold, 2021). Plants such as *Moringa oleifera*, *Trigonella foenum-graecum* (fenugreek) (Sevrin et al., 2019), and *Foeniculum vulgare* (fennel) contain bioactive phytochemicals, including phytoestrogens, flavonoids, and alkaloids (Alu'datt et al., 2024), which are believed to influence prolactin secretion, promote mammary gland activity, and enhance lactation outcomes. Initial evidence of their effectiveness has been presented through preclinical and clinical investigations (Gavahian et al., 2024). Fenugreek (*T. foenum-graecum* L.) has been recognized as a herbal galactagogue for centuries. Several studies have explored its active compounds and mechanisms of action contributing to its efficacy. Fenugreek seeds are rich in bioactive compounds such as steroidal saponins, flavonoids, alkaloids, and polyphenols. Key compounds include diosgenin, galactomannan, and various flavonoid glycosides. Diosgenin, a steroidal saponin, has notable physiological actions, including hormone synthesis. Galactomannan, a polysaccharide, contributes to fenugreek's physicochemical properties, providing thickening and stabilizing functions beneficial for food preparation and nutrition (Dhull et al., 2023; Prema, Justin Thenmozhi, Manivasagam, Mohamed Essa, & Guillemin, 2017). Galactomannan may also influence lactogenic effects by affecting the digestive system and glucose metabolism, which are indirectly related to lactation efficiency (Sevrin et al., 2020). Additionally, *Moringa oleifera* has been studied for its potential to increase breast milk production. It is a rich source of antioxidants, vitamins such as A and C, and essential amino acids, all of which support lactation (Klimek-Szczykutowicz et al., 2024). Its pharmacological activities, including anti-inflammatory and antioxidative properties, can be beneficial in managing the increased metabolic demands during lactation (Dhongade, Paikra, & Gidwani, 2017).

On the other hand, *Polyscias scutellaria* or shield aralia, known locally in Indonesia as "*daun mangkokan*," is a native herbal medicine belonging to the family Araliaceae, which is located in tropical regions of Southeast Asia and the Pacific Islands. *P. scutellaria* contains various bioactive compounds such as flavonoids, terpenoids, saponins, tannins, phenolic acids, and essential oils. Ethanolic extract of *P. scutellaria* has been reported for its anti-inflammatory effects on lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 cell lines (Nguyen et al., 2025). *P. scutellaria* leaves exhibit significant antioxidant activities due to bioactive compounds that can scavenge free radicals and reduce oxidative stress (Nasution, Ginting, & Lister, 2022). *P. scutellaria* has also been explored for herbal galactagogue properties. Indonesian society typically consumes *P. scutellaria* as a decoction. Previous scientific research revealed that the aqueous extract of shield aralia leaves can elevate prolactin and oxytocin levels in lactating rats over a three-

week period, continuing until the weaning stage. The bioactive compounds from *P. scutellaria*, including afzelin, kaempferol, quercetin, quercitrin, and rutin, can act as dopamine D<sub>2</sub> receptor inhibitors and 5-hydroxytryptamine 2A receptor (5-HT<sub>2A</sub>R) inducers. These mechanisms increase prolactin and oxytocin levels, thus promoting breast milk production (Budiono et al., 2023). However, the aqueous extract of *P. scutellaria* has a strong odor, which causes low interest among breastfeeding mothers. This issue has gained interest in developing functional foods fortified with *P. scutellaria* leaves.

Yogurt is one of the functional foods, made from milk fermented by lactic acid bacteria (LAB). Fortification in yogurt products is believed to increase their value and can also be used as herbal galactagogues. Previous studies have reported that yogurt infused with *P. scutellaria* leaves resulted in the formation of new bioactive compounds, namely hydroxy-octadecanoic acid (9-HpODE), (15Z)-9,12,13-trihydroxy-15-octadecanoic acid, 13(S)-hydroperoxylinolenic acid (13(S)-HPOTrE), and (E)-6-hydroxyoctadec-4-enoic acid (NP-011548), which belong to  $\alpha$ -linolenic acid (ALA) (Pertami et al., 2024). Further research is needed to provide valid data regarding the galactagogues properties of yogurt infused with *P. scutellaria* leaves, using animal experimental research. This study aimed to evaluate the galactagogues mechanisms of yogurt infused with *P. scutellaria* in lactating rats.

## 2. MATERIAL AND METHODS

### 2.1. Plant Collection and Yogurt Production

Fresh shield aralia (*P. scutellaria*) leaves were obtained from Materia Medica Batu (MMB), Batu City, East Java, Indonesia. 3% of fresh *P. scutellaria* leaves were blended with fresh milk. The mixture was then added with 6% sucrose, followed by pasteurization at 85 °C for 30 minutes (Rifa'i et al., 2025). The pasteurized milk was cooled to approximately 40 °C. Subsequently, 2% yogurt starter (Biokul Greek-yogurt, PT. Jaya Utama Santikah, Indonesia) was added, and the mixture was incubated for 24 hours at 37 °C (Pertami et al., 2024). After incubation, the yogurt was stored in a chiller for further analysis.

### 2.2. Experimental Design in Lactating Rats

Before starting the in vivo study, the animal welfare and experimental procedures methods have been approved by the Ethics Committee of Brawijaya University with approval number 198-KEP-UB-2023.

Twenty-five unmated female Wistar rats (180±20 grams of body weight, 8±2 weeks old) and four male Wistar rats with similar characteristics were acclimatized for seven days prior, given a standard chow diet, and *ad libitum* drinking water. After acclimatization, the female rats (n=20) were fertilized with the male rats (n=4). Fertilization was performed by placing five female rats with a male rat in a cage. After fertilization, the female rats were housed individually and monitored throughout the gestation period, which lasted approximately 21-24 days. Following parturition, during the lactation stage, the female rats were divided into five groups: unmated rats (UR), negative control lactating rats (NCR), positive control lactating rats (PCR) treated with domperidone (using human to animal dose conversion), lactating rats treated with *P. scutellaria* yogurt at a dosage of 2 g/kg BW (LRPSY1), and lactating rats treated with *P. scutellaria* yogurt at a dosage of 4 g/kg BW (LRPSY2). The number of replications in animals was calculated using Federer's formula with Equation 1.

$$(t - 1)(n - 1) \geq 15 \quad (1)$$

Where:

n = Number size of each intervention/replication.

t = Number of treatment groups.

According to Federer's formula, the minimum number of replications in each group was 5 mice.

Treatments were administered orally during the lactation stage (21 days). Blood samples were collected weekly for prolactin (PRL) and oxytocin (OXT) assays. The body weights of dams and pups were measured weekly during

the lactation period. After treatment, the dams were euthanized using ketamine (100 mg/kg BW) and xylazine (10 mg/kg BW).

### 2.3. Prolactin and Oxytocin Assay

The weekly levels of prolactin (PRL) and oxytocin (OXT) were measured using enzyme-linked immunosorbent assay (ELISA). Blood from the dams was collected weekly for three weeks until weaning. During the first and second weeks, blood was drawn from the lateral tail vein, while in the third week, it was collected via cardiac puncture. The collected blood was centrifuged at 2500 rpm for 20 minutes at 10°C, after which the serum was extracted for the measurement of PRL and OXT levels. The PRL assay utilized a rat prolactin ELISA kit with catalogue no. EA0020Ra (BT-LAB, Shanghai, China), while the OXT assay used a rat oxytocin ELISA kit with catalogue no. EA0071Ra (BT-LAB, Shanghai, China). The sensitivity of the rat prolactin ELISA kit was 0.3 ng/mL, whereas the rat oxytocin ELISA kit had a sensitivity of 2 ng/mL.

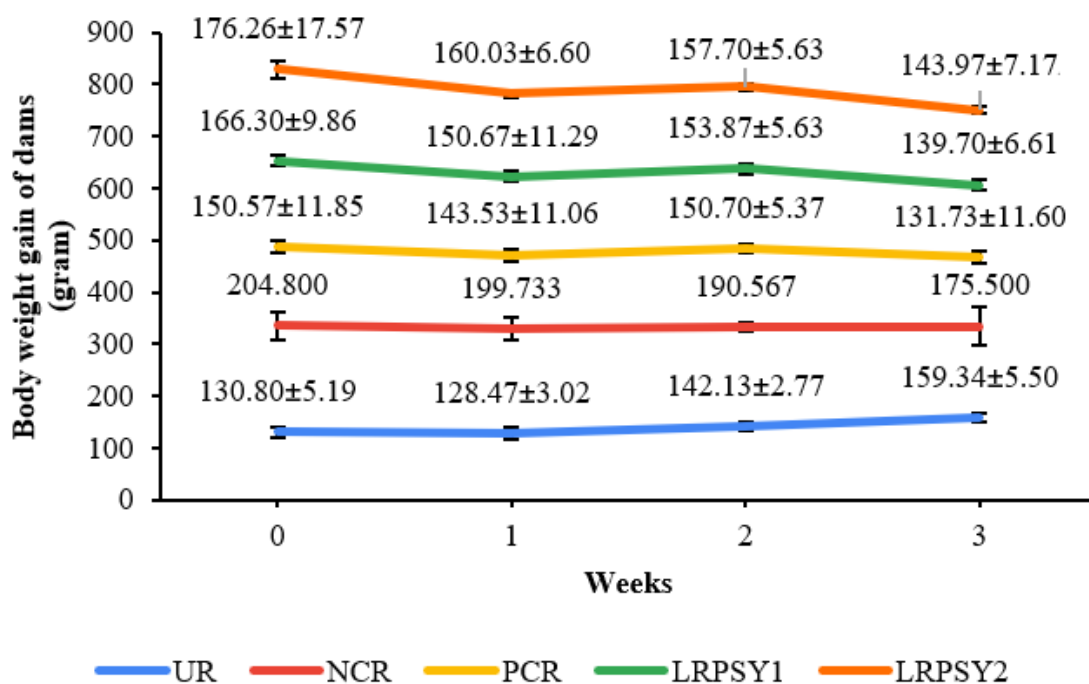
### 2.4. Data Analysis

Data were presented as mean  $\pm$  standard deviation of the mean. The weekly PRL and OXT data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan Multiple Range Test (DMRT) as a post hoc test, with  $P < 0.05$  indicating significant differences between groups.

## 3. RESULTS

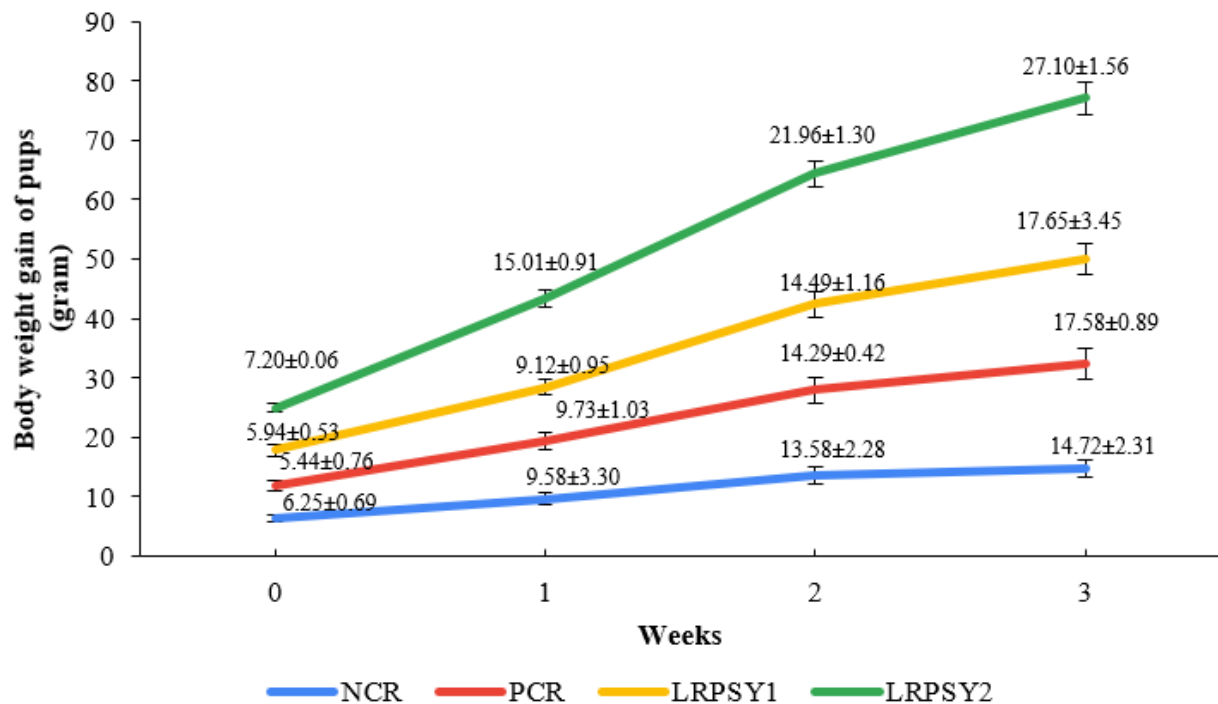
### 3.1. Supplementation of *P. Scutellaria* Yogurt in Body Weight of Dams and Pups

Based on Figure 1, it was shown that the UR group increased in body weight over a three-week observation period. Meanwhile, the body weight of lactating rats continued to decrease during the lactation stage. This mechanism could be caused by the lactation process requiring much energy, which makes the body weight of breastfeeding mothers lower.



**Figure 1.** The body weight gain of dams for 3 weeks before weaning. UR, unmated rats; NCR, negative control rats; PCR, positive control rats; LRPSY1, lactating rats treated with *P.scutellaria* yogurt 2 g/kg BW; LRPSY2, lactating rats treated with *P.scutellaria* yogurt 4 g/kg BW. The data presented as mean $\pm$ standard deviation.

Based on the Figure 2, it showed that the NCR group has the slowest gain in the body weight of pups (blue line). Meanwhile, supplementation of *P.scutellaria* yogurt for dams during lactation increases the body weight gain of pups compared with the NCR group. Supplementation of *P.scutellaria* yogurt at a dosage of 4 g/kg BW has the highest body weight gain among the groups.



**Figure 2.** The body weight gain of pups over the 3 weeks before weaning. UR, unmated rats; NCR, negative control rats; PCR, positive control rats; LRPSY1, lactating rats treated with *P.scutellaria* yogurt 2 g/kg BW; LRPSY2, lactating rats treated with *P.scutellaria* yogurt 4 g/kg BW. The data are presented as mean  $\pm$  standard deviation.

### 3.2. *P. Scutellaria* Yogurt Able to Increase Weekly Prolactin and Oxytocin During Lactation Stage

The data analysis presented in Table 1 indicates that prolactin levels were lowest in unmated rats compared to other groups. Since unmated rats do not undergo lactation, their prolactin and oxytocin levels are naturally low. The NCR group, consisting of lactating rats without treatment, showed a significant increase in prolactin levels compared to the UR group. However, the NCR group still had the lowest prolactin levels among the lactating rat groups. The PSR group, which received domperidone treatment, demonstrated an increase in prolactin levels compared to the NCR group. Additionally, the supplementation of *P.scutellaria* yogurt during lactation contributed to an increase in prolactin levels. Weekly evaluations of prolactin levels revealed that by the third week of lactation, *P.scutellaria* yogurt resulted in higher prolactin levels compared to domperidone. These findings suggest that *P.scutellaria* yogurt may serve as a functional food with galactagogue properties.

**Table 1.** The weekly prolactin serum levels in lactating rats for a 3-week lactation period.

Treatment group	Prolactin levels		
	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week
UR	5.649±0.75 <sup>a</sup>	15.526±0.71 <sup>a</sup>	31.253±0.74 <sup>a</sup>
NCR	9.6717±1.45 <sup>b</sup>	23.377±8.18 <sup>a</sup>	50.231±9.76 <sup>b</sup>
PCR	34.7413±8.97 <sup>c</sup>	56.248±9.62 <sup>b</sup>	83.557±5.11 <sup>c</sup>
LRPSY1	29.465±6.91 <sup>c</sup>	47.397±6.69 <sup>b</sup>	150.175±1.14 <sup>e</sup>
LRPSY2	31.353±8.17 <sup>c</sup>	47.577±3.14 <sup>b</sup>	114.556±3.65 <sup>d</sup>

**Note:** UR, unmated rats; NCR, negative control rats; PCR, positive control rats; LRPSY1, lactating rats treated with *P.scutellaria* yogurt 4 g/kg BW; LRPSY2, lactating rats treated with *P.scutellaria* yogurt 2 g/kg BW. The data are presented as mean  $\pm$  standard deviation and were analyzed using ANOVA followed by DMRT as a post hoc test. <sup>a,b,c,d,e</sup> Different letters indicate significant differences ( $p < 0.05$ ).

Based on the data analysis on oxytocin levels (Table 2), it shows that the UR group has the lowest oxytocin level during the three-week lactation stage. On the other hand, the NCR group has the lowest oxytocin levels among lactation rat groups. Furthermore, supplementation with *P.scutellaria* yogurt demonstrates higher weekly oxytocin levels compared with the PCR group, which is treated with domperidone. These results indicate that *P.scutellaria* yogurt increases weekly oxytocin levels during the lactation stage.

**Table 2.** The weekly oxytocin serum levels in lactating rats during a 3-week lactation period.

Treatment group	Oxytocin levels		
	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week
UR	0.777±0.10 <sup>a</sup>	5.554±0.89 <sup>a</sup>	5.949±0.61 <sup>a</sup>
NCR	2.752±0.55 <sup>b</sup>	5.311±0.66 <sup>a</sup>	6.293±0.95 <sup>a</sup>
PCR	4.655±1.37 <sup>c</sup>	19.851±1.39 <sup>c</sup>	22.046±1.71 <sup>b</sup>
LRPSY1	11.841±1.35 <sup>d</sup>	15.001±1.50 <sup>b</sup>	33.882±3.96 <sup>c</sup>
LRPSY2	13.881±0.62 <sup>e</sup>	15.638±3.75 <sup>b</sup>	35.332±3.95 <sup>c</sup>

**Note:** UR, unmated rats; NCR, negative control rats; PCR, positive control rats; LRPSY1, lactating rats treated with *P.scutellaria* yogurt 2 g/kg BW; LRPSY2, lactating rats treated with *P.scutellaria* yogurt 4 g/kg BW. The data are presented as mean ± standard deviation and were analyzed using ANOVA followed by DMRT as a post hoc test. <sup>a,b,c,d,e</sup> Different letters indicate significant differences ( $p < 0.05$ ).

#### 4. DISCUSSION

Hypogalactia is characterized by insufficient breast milk production to satisfy the nutritional needs of infants. It continues to be a significant clinical and public health issue. This condition may result from various etiologies, including maternal endocrine disorders, inadequate development of the mammary glands, retained placental fragments, impaired prolactin release, or psychosocial stressors (Sandoval-Pinto, García-Gutiérrez, Acosta-Real, Sierra-Díaz, & Cremades, 2023; Zhdanova, Malinovskaya, & Yunusova, 2018). The complex etiology of hypogalactia presents challenges in its management, often requiring an integrated strategy that combines medical, nutritional, and behavioral interventions (Raji, Oriji, & Ahmad, 2023). Numerous medicinal plants have been studied for their galactagogue properties. The bioactive compounds found in galactagogue plants, including phytoestrogens, flavonoids, and saponins, are believed to promote lactation by modulating endocrine functions (Kelleher, Burkinshaw, & Kuyooro, 2024). Phytochemicals with estrogenic activity can enhance ductal branching and alveolar budding, thereby expanding the secretory capacity of mammary tissue (Foong et al., 2020).

Furthermore, yogurt as a functional food product has been reported to provide benefits for breastfeeding mothers. The consumption of yogurt during the lactation period offers several advantages for both mothers and their infants. Notably, yogurt is a substantial source of calcium, which is essential for maintaining maternal bone integrity and supporting the skeletal growth (Hadjimbei, Botsaris, & Chrysostomou, 2022) of the breastfeeding infant. This is particularly significant during lactation, as the demand for calcium increases to meet the requirements of milk production. Yogurt is not only rich in calcium but also provides a significant amount of protein, which is crucial for the repair and growth of body tissues (Hadjimbei et al., 2022). Research on lactating women has demonstrated that increasing maternal dietary protein significantly boosts milk production while maintaining its nutritional quality, thereby supporting adequate infant growth and development. Additionally, yogurt contains probiotics, which can enhance gut health (Sayar & Köseoğlu, 2025). Probiotics may improve the mother's digestive health and could also influence the infant's gut flora when passed through breast milk (Qi et al., 2022), potentially strengthening the infant's immune system and reducing the risk of gastrointestinal infections (Gonia et al., 2024; Zaidi, Moore, & Okala, 2021).

This study's findings revealed that supplementation of *P.scutellaria* yogurt in lactating rats significantly increased the body weight gain of pups compared with NCR and PCR groups. The increase in body weight could be attributed to the higher quality and quantity of breast milk in rats supplemented with *P.scutellaria* yogurt compared to other lactating rat groups. This result is also supported by data on weekly prolactin and oxytocin levels in lactating rats, where supplementation with *P.scutellaria* yogurt enhanced the levels of prolactin and oxytocin hormones involved in breast milk production significantly compared to NCR and PCR groups. Previous studies have reported that

*P.scutellaria* leaves extract contains several bioactive compounds with galactagogue properties, such as kaempferol, quercetin, afzelin, quercitrin, and rutin (Budiono et al., 2023). These bioactive compounds have also been investigated for their role as inhibitors of dopamine D<sub>2</sub> receptors, which are involved in breast milk production pathways. Recent molecular docking and in vitro studies suggest that compounds like quercetin, kaempferol, and diosgenin interact with estrogen receptors (ER $\alpha$  and ER $\beta$ ) and prolactin receptors, modulating downstream signaling pathways such as JAK2/STAT5, which are essential for lactation-specific gene expression (Frasor, Barkai, Zhong, Fazleabas, & Gibori, 2001; Tian et al., 2020). Furthermore, other bioactive compounds found in *P.scutellaria* yogurt, belonging to ALA, include hydroxy-octadecanoic acid (9-HpODE), (15Z)-9,12,13-trihydroxy-15-octadecanoic acid, 13(s)-hydroperoxylinolenic acid (13(S)-HPOTrE), and (E)-6-hydroxyoctadec-4-enoic acid (NP-011548), which may contribute to galactagogue properties. Mazurier et al. (2017) demonstrated that supplementation of lactating women's diets with omega-3 PUFAs, such as ALA, increased their concentration in human milk. The study outlined that the ALA content was significantly augmented following a supplementation period. ALA, as an omega-3 fatty acid, plays an essential role in the composition of breast milk by contributing to the overall nutritional quality important for infant development.

On the other hand, functional food interventions during lactation have significant implications for both maternal and infant health, given the potential of such foods to enhance nutritional status and overall health outcomes (Essa et al., 2023). Furthermore, the bioavailability and solubility of functional food factors are critical challenges in delivering precision nutrition during lactation (Yu, Abd El-Aty, Su, & Tan, 2023). Advances in targeted delivery systems are becoming a focal point to improve these attributes, thereby enhancing the efficacy of functional food interventions (Yu et al., 2023). Pertami et al. (2024) has reported on developing a functional food for lactation in the form of lactation cookies. These lactation cookies are enriched with *P.scutellaria* leaves powder and possess antioxidant properties. Antioxidant and anti-inflammatory properties may protect mammary epithelial cells from oxidative stress, which is known to impair lactogenesis. Some studies suggest that herbal galactagogues could impact metabolic pathways, potentially influencing lipid and glucose metabolism, which might contribute to enhanced milk production (Sevrin et al., 2019). *P.scutellaria* yogurt can also be defined as a functional food with galactagogue properties. Yogurt enriched with *P.scutellaria* leaves has been reported for its nutritional content using proximate analysis, where the protein content in *P.scutellaria* yogurt reached 15.46%, indicating that this yogurt is categorized as a high-protein product (Pertami et al., 2024). Yogurt is a convenient and versatile food that can be easily incorporated into a lactating mother's diet, providing a quick source of energy and nutrition. It supports the overall nutritional requirements of lactating women, which is vital for maintaining optimal milk production and ensuring that the composition of breast milk meets the infant's nutritional needs. The composition of breast milk, which can be influenced by maternal nutritional interventions, plays a vital role in the developmental programming of the infant, including organ differentiation and metabolic programming during the early postnatal period (Rodríguez-González, Bautista, Rojas-Torres, Nathanielsz, & Zambrano, 2020).

## 5. CONCLUSION

This study reported that supplementation of *P.scutellaria* yogurt enhanced the quality and quantity of breast milk production based on the body weight gain of pups, weekly levels of prolactin and oxytocin from dam rats. The body weight gain of pups with the dams supplemented with *P.scutellaria* yogurt is higher than in other lactating rat groups. The weekly prolactin and oxytocin levels are also higher in lactating rats supplemented with *P.scutellaria* yogurt compared to breast milk booster drugs. These findings suggest that supplementation of *P.scutellaria* yogurt during lactation can help improve the quality and quantity of breast milk production. Further research is recommended to evaluate the efficacy of *P.scutellaria* yogurt in humans, especially in breastfeeding mothers.

**Funding:** This research was supported by the Ministry of Health Republic of Indonesia with (Grant number DP.04.03/F.XXI/1718/2024).

**Institutional Review Board Statement:** The Ethics Committee of Brawijaya University, Indonesia has granted approval for this study on 23 November 2023 (Ref. No.\_198-KEP-UB-2023).

**Transparency:** The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.

**Competing Interests:** The authors declare that they have no competing interests.

**Authors' Contributions:** All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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