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Characteristic Nutritional Composition, Protein Digestibility, and Total Lactic Acid Bacteria of Plant-Based Yogurt from Different Legume

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ABSTRACT

Several types of local Indonesian legume have the potential to be developed into plant-based milk yogurt, including pigeon peas, soybeans, and mungbeans. However, the protein contained in legume milk is characterized by low digestibility and bioavailability, underscoring the need to combine several types of legume and proper bioprocessing. Fermentation of legume milk by *L. bulgaricus* and *S. thermophilus* has been shown to improve the sensory and nutritional value of yogurt. Therefore, this study aims to analyze the nutritional value, protein digestibility, and total LAB of yogurt from a single type of legume (pigeon peas) and a combination (pigeon peas, mungbeans, and soybeans). The experiment used a completely randomized design with one factor, namely the composition of each legume, consisting of 4 treatments and 5 repetitions. The treatment of the study consisted of P1 (pigeon peas), P2 (pigeon peas + mungbeans 1:1), P3 (pigeon peas + soybeans 1:1), and P4 (pigeon peas + mungbeans + soybeans 1:1:1). The results showed that composition of legume type had a significant effect on proximate levels, dissolved protein, protein digestibility, lactic acid, and pH, but not the total lactic acid bacteria LAB of yogurt. The protein content, digestibility, and the soluble protein of legume-yogurt increased by approximately 1.21-1.86%, 0.36-0.44%, and 0.81-1.30%, respectively. In conclusion, yogurt from a single type of legume produced lower proximate levels, dissolved protein, protein digestibility, lactic acid, and low pH, while the combination of two or three types showed higher values for these parameters. Among the combinations, pigeon peas yogurt + soy produced higher proximate, dissolved protein, protein digestibility, lactic acid, as well as lower pH compared to pigeon peas yogurt + mungbeans.

Keywords: Pigeon peas; mungbeans; soybeans; legume protein; plant-based yogurt

INTRODUCTION

Legume is considered plant-based foods with diverse nutritional content, including protein, dietary fiber, carbohydrates, minerals, and vitamins. Functional properties such as emulsifiers and gel-formers make legume suitable for producing non-dairy yogurts (Jamalullail et al., 2023). Among local Indonesian legume, pigeon peas, soybeans, and mungbeans have strong potential to be developed into plant-based yogurt. Pigeon peas are underutilized despite the potential to be novel protein sources of lysine, albumin, globulin, methionine, tryptophan, and prolamin, as well as essential macro and micro nutrients (vitamin B, C, and carotene) (Raharjo et al., 2021). Soybeans are well known as a protein source, providing amino acids,

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including cystine, lysine, leucine, isoleucine, arginine, histidine, methionine, phenylalanine, threonine, valine, and tryptophan. In addition, soyabean contain fats such as palmitate, stearate, oleate, linoleic, and arachidic. It is also composed of carbohydrates, vitamins (E, thiamine, riboflavin, pantothenic acid, biotin, folic acid, and niacin), and minerals (Ca, P,Mg, K, and Na) (Hany El-Shemy, 2011).

Mungbeans contain complete macro and micronutrients such as carbohydrates, proteins (alanine, arginine, lysine, leucine, isoleucine, glycine, serine, histidine, methionine, phenylalanine, proline, valin, aspartic acid, cysteinic acid, glutamic acid, and tryptophan), fats (palmitate, stearate, oleic, linoleic and behenic acid), fiber, vitamins (thiamine, riboflavin, niacin and vitamin C) and minerals (calcium, copper, iron, magnesium, potassium, sodium, phosphate and zinc) (Dahiya et al., 2015).

Despite the nutritional richness, legume milk protein is characterized by low digestibility and bioavailability due to a low amount of essential amino acids and excess dietary fiber, as well as the presence of antinutritional factors, including phytic acid, tannins, and trypsin inhibitors (Rashwan et al., 2023). Lactic Acid Fermentation in legume has been shown to promote concentration of free amino acids, peptides, soluble fiber, and total phenols, while also increasing protein digestibility and nutritional value (Montemurro et al., 2021). Lactic Acid Bacteria (LAB) are capable of breaking down legume carbohydrates from glucose and hexose types to produce digestible protein (Nugroho et al., 2023). LAB increases protein digestibility of yogurt by reducing anti-nutritional factor (ANF) (Montemurro et al., 2021). The combination of *L. bulgaricus* and *S. thermophilus* in legume fermentation reportedly improved sensory properties, nutritional value, and provided health benefits (Emkani et al., 2022). Fermentation by combining several types of legume can be a strategy to increase protein content and digestibility.

Plant-based yogurt is expected to have texture, sensory characteristics, and nutritional properties similar to conventional dairy milk yogurt (Demir et al., 2023). Yogurt from pigeon bean, soybeans, and mungbeans has a high nutritional value, contains bioactive components (such as phenolics, flavonoids, procyanidin, and non-allergenic protein), dietary fiber, essential fatty acids, minerals, and vitamins (Rashwan et al., 2023). Many studies of plant-based yogurt from legume (pigeon peas, soybeans, and mungbeans) have been conducted. In a previous study, pigeon peas (30%) and beet tuber extract (15%) formulations produced yoghurt with the highest antioxidant activity, while pigeon peas (20%) and beet tuber extract (15%) formulations yielded acceptable sensory characteristics and quality nutritional content (Prehatin et al., 2020). Moreover, pigeon pea milk substitution with *Annona muricata* juice (10%) also produced yogurt, which had acceptable sensory properties and good nutritional value (Oresanya et al., 2022).

Formulated legume yogurt (soybean, mungbeans, pigeon peas flour, and milk) has been reported as an alternative to fermented dairy milk with large probiotic bacterial viability during storage (> 7 log cfu/mL). Yogurt produced from legume milk tends to have a higher nutrient composition than flour (Jamalullail et al., 2023). The combination of mungbean milk and soybean milk had a significant effect on total solids and protein content but not total acidity, pH, color, aroma, flavor, and consumer acceptance (Yusdianti et al., 2014)

Based on literature reviews, no study has examined the nutritional content and digestibility of yogurt protein produced from a combination of pigeon pea, soybean, and mungbean milk. Therefore, this study aims to evaluate nutritional value (proximate), protein digestibility, and total LAB of yogurt produced from pigeon peas or a combination of pigeon peas, mungbeans, and soybeans. The results are expected to provide an alternative protein-rich yogurt from local legume with good protein digestibility.

METHODS

Materials

This study used a mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* isolates obtained from FNCC Gajah Mada University. De Man,Rogosa, and Sharpe (MRS) agar (Oxoid) was used as the growth medium. The legume used in this study was mungbeans, yellow soybeans (Anjasmoro variety) harvested at 3 months in Maguwoharjo, Sleman, Yogyakarta, and white pigeon peas (Jenangan, Ponorogo, East Java Province). Other materials were sucrose from gulaku, distilled water, bovine serum albumin (BSA; Sigma-Aldrich), Lowry B reagent (Nitra Kimia Laboratory), Lowry A reagent (Nitra Kimia Laboratory), NaOH 0.1 N (Sigma-Aldrich), and phenolphthalein solution (Merck).

Some of the instruments used include a biosafety cabinet (Infitek), autoclave (All American 75X), incubator (Memmert), oven (Modena), Soxhlet (Pyrex), spectrophotometer (Shimatsu), waterbath (Memmert), spectrophotometer (Shimatsu), rotary evaporator (B-One), and colony counter (B-One). Others were a pH meter (Hanna), burettes and pipettes (Pyrex), titration measuring instruments (Hanna), erlenmeyer 250 mL, a glass bottle with a capacity of 1000 mL, a thin cotton cloth, a strainer, and silica gel.

Experimental Design

A complete random design was used with one factor, namely the legume type, with four treatments and five repetitions. The treatments include P1 = pigeon peas milk, P2 = pigeon peas +mungbeans milk ratio 1:1, P3 = pigeon peas + soybeans milk ratio 1:1, and P4 = pigeon peas + mungbeans + soybeans milk ratio 1:1:1. Data were statistically analyzed using one-way Anova test, and when significant difference occured, Duncan Multiple Range Test (DMRT) test was performed at 5% significant level using SPSS 23.0 version.

Preparation of Pigeon Peas, Mungbean, and Soybean Milk

Pigeon pea, soybean, and mungbean were sorted, washed, and steamed for 10 minutes separately, then soaked for 24 hours. After soaking, the legume were rinsed and blended separately with distilled water at 70 °C using a legume-to-water ratio of 1:3. For soybeans, the seed coats were removed before blending. The pulp was filtered using a thin cotton cloth, and the supernatant was pasteurized at a temperature of 82 °C for 15 minutes (Oresanya et al., 2022; Gyeongseon et al., 2024, with modification). After pasteurization, pigeon peas, soybeans, and mungbeans milk were obtained at a temperature of 50 °C.

Inoculum Preparation

Mixed cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were activated by using the pour plate method on deMan Rogosa and Sharp Agar (MRSA) media. Approximately 0.10 g of mixed isolated powder was swabbed with a cotton bud into MRSA and incubated at 37 °C for 24 hours to obtain an active culture. A suspension culture was then prepared by inoculating one loopful (ose) of the active culture into 100 mL of MRS broth and incubating at 37 °C for 24 hours, following the method of (Kadyan et al., 2021) with slight modifications.

Preparation of Starter Solution

About 10 mL of the culture suspension solution was added to legume milk according to the treatment until the total volume reached 100 mL. The mixture was incubated at 37 °C for 8 hours, and the cell population was calculated using the Total Plate Count (TPC). When the culture reached the exponential growth phase with a cell density of approximately 10^7 CFU/mL, it was used as the starter solution (Elghali et al., 2014).

Fermentation Steps

Approximately 90 mL of legume milk of various types, according to the treatment, was pasteurized using a water bath at 82 °C for 15 minutes and cooled until it reached a temperature of 42 °C. Sucrose (5%) was added, followed by inoculation with a starter solution of 2% (b/v) containing approximately 10^7 log CFU/mL. The mixture was further incubated at 37 °C for 8 hours (ModKolawole et al., 2015; Rana et al., 2021; Winarsi et al., 2021; Oresanya et al., 2022). The formulation of yogurt from various types of legume is summarized in Table 1.

Proximate Measurement

Proximate measurement included determination of carbohydrates, protein, fat, moisture content, and ash. Moisture content was analyzed using gravimetry, where the sample was oven-dried at 105-110 °C for 2-5 hours until a constant weight was obtained (AOAC method 977.11). Ash content was examined by burning the sample in a furnace at 550-600 °C for 12-24 hours (AOAC method 923.03). Total protein levels were measured using the Kjeldahl method (AOAC method 955.04), while fat content was assessed through the Soxhlet method (AOAC method 960.39). Finally, carbohydrate content was calculated using Luff school method (Othman et al., 2019).

Soluble Protein Content Testing

Soluble protein contents were determined using the Lowry method (Lowry et al. 1951). About 1 mL of the yogurt sample was centrifuged at 5,000 rpm for 10 min, and the supernatant was used for analysis. A total of 0.5 mL supernatant was mixed with 2.5 mL of Lowry reagent (mixture of Lowry A and Lowry B reagents), followed by incubation for 10 min at room temperature. Subsequently, 0.25 mL of Folin–Ciocalteu phenol reagent (diluted 1:1 with distilled water) was added, and the mixture was vortexed and incubated for 30 min in the

Table 1. Yogurt formulation from different types of legume

Milk formulation	P1	P2	P3	P4
Pigeon peas milk (mL)	90	45	45	30
Mungbeans milk (mL)	0	45	0	30
Soybean milk (mL)	0	0	45	30
Sucrose (g)	5	5	5	5
Starter (mL)	2	2	2	2

dark. The absorbance was measured at 750 nm using a spectrophotometer. Bovine serum albumin (BSA) was used as the standard, and soluble protein content was expressed in mg BSA equivalent per mL sample.

Protein Digestibility Testing

Protein digestibility was measured using the in vitro multienzyme method (Demir et al., 2023) as described by Demir et al. (2023) with slight modifications. About 10 mL of yogurt sample was adjusted to pH 8.0 using 0.1 N NaOH and maintained at 37 °C. A multienzyme solution containing trypsin, chymotrypsin, and pancreatin was prepared in phosphate buffer (pH 8.0). The digestion was initiated by adding 1 mL of enzyme solution to the sample while continuously stirring. The pH decrease was recorded for 10 min using a calibrated pH meter. Protein digestibility (%) was calculated based on the pH drop using the equation: Digestibility (%) = 210.46 – 18.10 × (pH at 10 min).

pH

pH was measured using a pH meter that has been calibrated using buffer 4 (Priyadarshani & Muthumuniarachchi, 2018).

Lactic Acid Content Testing

Lactic acid level was measured using the titration method and expressed as the percentage of lactic acid (Udensi et al., 2023).

Total Lactic Acid Bacteria

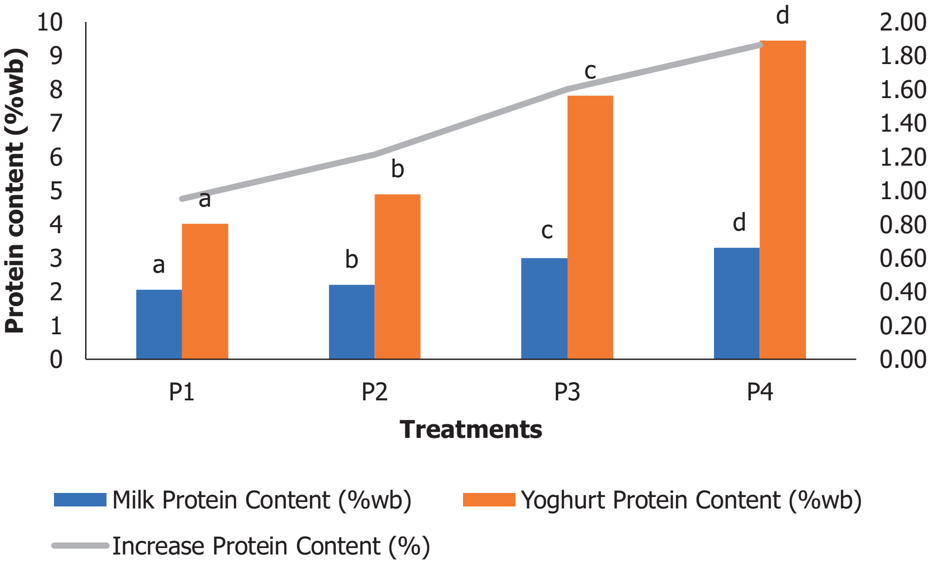
Total LAB was measured using the pour plate method on de Man, Rogosa, and Sharpe Agar (MRSA) following Nugroho et al. (2023). About 1 mL of the yogurt sample was homogenized with 9 mL of sterile 0.85% NaCl to obtain a 10^{-1} dilution, followed by serial decimal dilutions up to 10^{-7}. An aliquot of 1 mL from appropriate dilutions was poured into sterile Petri dishes, and approximately 15–20 mL of molten MRSA (45 °C) was added. The plates were gently mixed and allowed to solidify, then incubated at 37 °C for 48 h under anaerobic conditions. Colonies were counted from plates containing 30–300 colonies, and results were expressed as log CFU/mL of yogurt.

RESULTS AND DISCUSSION

Total Protein

Total Protein represents the combined protein content derived from yogurt substrates and protein of LAB (starter). As shown in Figure 1, milk and yogurt produced from a single legume type had lower protein levels, while products formulated from combinations of different legume types had higher protein contents.

The result showed that the legume type had a significant effect ($p < 0.05$) on milk total protein levels. The protein content from various types of legume milk ranged from 2.06 to 3.30%wb. The variation in milk



Note: superscripts on bar charts with the same color indicated significant differences in the One-Way ANOVA test with significance level of 5% ($p < 0.05$). P1 = pigeon peas; P2 = pigeon peas + mungbeans; P3 = pigeon peas + soybeans; P4 = pigeon peas + soybeans+ mungbeans, n= 5 repetitions.

Figure 1. Comparation of total protein content in legume milk and legume yogurt

protein content can be attributed to the presence of protein with different types of amino acids in pigeon peas, mungbeans, and soybeans. The standard procedure for total protein measurement using the Kjeldahl method requires the digestion of food with a strong acid, leading to the release of nitrogen, which is then quantified using a titration technique. Protein quantity is then calculated from the nitrogen concentration of the sample using a conversion factor (usually 6.25, which is equivalent to 0.16 g nitrogen per gram of protein). Given that all amino acids contain at least one amine group with nitrogen, an increase in nitrogen-rich amino acids contributes to higher measured total protein values.

According to the Food and Agriculture Organization of the United Nations (FAO), protein quality depends on both the amino acid composition and bioavailability, which is closely related to digestibility (Hayes, 2020). Therefore, the combination of pigeon peas with other types of legume can result in a higher protein content (Raharjo dkk., 2021). Pigeon peas contain 18-35% protein with 20-22% in the form of the amino acid lysine (Febriani et al., 2019). Various types of amino acids often found include aspartic acid, lysine, leucine, arginine, glutamic acid, methionine, and cystine (Singh et al., 2022). Pigeon peas also consist of albumin, globulin, prolamin, and glutelin. Other types of amino acids found are histidine, isoleucine, phenylalanine, threonine, valine, alanine, glycine, proline, serine, tryptophan, and tyrosine.

Soybeans contain about 40% protein, mainly β-concycycine and glycine (Gyeongseon et al., 2024). The amino acid composition includes lysine, histidine, arginine, serine, cystine, proline, alanine, aspartate, threonine, glycine, glutamate, valine, methionine, leucine, isoleucine, tryosine, and phenylalanine (Raharjo et al., 2021). Mugbeans contain 20-24% protein dominated by globulin and albumin (Winarsi et al., 2021), as well as various amino acids, namely arginine, lysine, leucine, isoleucine, valine, aspartic acid, and phenylalanine (Agustina & Andriana, 2010; Amelia et al., 2016).

The composition of legume types had a significant effect ($p<0.05$) on the protein content of yogurt. Based on the results, yogurt from various types of legume contains protein ranging from 4.02 to 9.45%wb. The plant-based yogurts produced in this study met the requirements of SNI 2981:2009 for animal-milk yogurt protein content (minimum 2.7%) and, in several treatments, exceeded the protein levels typically reported for dairy-based yogurt.

Some of the factors that affect the protein content in yogurt include the amount of protein in the ingredients used, the amount of LAB, the legume grinding process, and the total dissolved solids (Purwijantiningsih, 2016). Fermentation using *S. thermophilus* and *L. bulgaricus* enhanced protein digestibility in yogurt (Nugroho et al., 2023). The yogurt protein data were consistent with milk protein and soluble protein results, showing that yogurt produced from a single legume type (pigeon peas) had the lowest protein content, while combinations of pigeon peas, mungbeans, and soybeans resulted in higher and variable protein levels.

During fermentation, LAB show proteolytic activity by hydrolyzing protein into smaller peptides and free amino acids (Lim et al., 2019). This process does not create new protein but increases the measurable fraction, especially soluble peptides, and together with a concentration effect may result in a higher protein value expressed on a wet-weight basis due to reduced water content and higher total solids after fermentation (Ter et al., 2024). The content and composition of yogurt protein change in response to protease activity through the mechanism of peptide bonding, producing free polypeptides or amino acids. In addition to protease activity, the organic acid component produced by LAB can also damage the secondary and tertiary structures of protein due to ionic interactions between the protein side chains. The greater the amount of protein sources easily fermented by LAB, the higher the protein content of yogurt produced (Emkani et al., 2022). The protein content is not only determined by the composition of amino acids, but also digestive rate, protein structure, and enzyme accessibility (Samben & Puspaningrum, 2020).

Fermentation did not increase the total amount of protein, because proteolysis converts protein into peptides and free amino acids without adding nitrogen to the system. Therefore, total protein determined by the nitrogen-based methods (Goulding et al., 2020) is expected to remain relatively constant unless a concentration effect occurs due to decreased moisture content or increased total solids. In contrast, the proteolytic system of LAB may increase soluble nitrogen and protein fraction (soluble peptides and amino acids), potentially resulting in higher values when protein is assessed using soluble-protein assays.

Soluble Protein

Soluble protein represents a fraction of total protein in food, which exists in the form of peptides or amino acids easily absorbed by the body (Winarsi et al., 2021). Higher soluble protein content is considered nutritionally advantageous. The soluble protein levels of legume milk and the corresponding yogurt products are presented in Figure 2. The results indicated that the milk and yogurt produced from a single legume type had the lowest soluble protein levels, while formulations

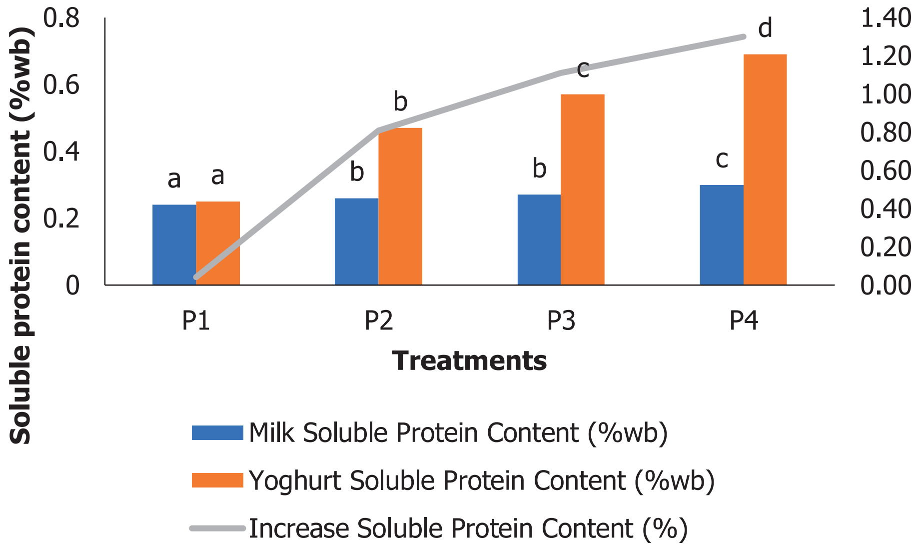


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Figure 2. Comparation of soluble protein content in legume milk and legume yogurt

derived from combinations of different legume types had increased soluble protein content.

Figure 2 shows that legume type had a significant effect ($p < 0.05$) on soluble protein content. Various types of legume milk contain soluble protein ranging from 0.24 to 0.30%wb. The higher soluble protein content observed in milk produced from combinations of legumes may be attributed to differences in amino acid composition among legume types. Protein solubility, which directly influences soluble protein content, is affected by amino acid composition, functional groups, and intramolecular and intermolecular bonds that determine protein structural conformation. In addition, intrinsic factors, including protein size and charge, as well as extrinsic factors, namely pH, ion strength, and temperature, also affect protein solubility (Hanley et al., 2025). However, this study did not measure the solubility of protein. Increase in soluble protein in legume milk is influenced by several factors, including the type of legume and the treatment stages in preparation. The composition of different protein fractions affects the level of protein solubility in water. Treatment stages such as soaking and grinding play a role in increasing hydration and cell wall damage, thereby facilitating protein release (Khattab et al., 2009).

The composition of the legume type has a significant effect ($p < 0.05$) on the soluble protein content. Yogurt from various types of legume contains soluble protein ranging from 0.25 to 0.69% wb. The increase in soluble protein content can be attributed to *L. bulgaricus and S. thermophilus* having proteolytic activity to hydrolyze protein into soluble amino acids with a smaller molecular weight, thereby affecting the level of dissolved protein (Purwantiningsih et al., 2022). Enzymatic activity of LAB hydrolyzes protein, causing changes in molecular weight, structural conformation, and increased solubility during fermentation, through protein decomposition and degradation induction. The increase in protein solubility affects the soluble protein level (Zhai et al., 2025).

Protein Digestibility

Protein digestibility reflects the extent to which proteins can be hydrolyzed by proteolytic enzymes into absorbable amino acids for utilization by the body. Several factors affect the digestibility value of protein, including processing methods, the presence of antinutrient compounds, and the reaction between protein and other compounds contained in food (Annisaa & Afifah, 2015). As shown in Figure 3, milk protein from single legume yogurt had lower digestibility, while the combination of legume types increased digestibility.

Figure 3 shows that legume type had a significant effect ($p < 0.05$) on protein digestibility value. Milk of various legume types was found to have protein digestibility ranging from 60.34 to 62.27%wb. The presence of anti-nutritional compounds in both pigeon peas, mungbeans, and soybeans caused a low protein digestibility value in milk. Pigeon peas contain anti-nutritional compounds in the form of phytic acid,

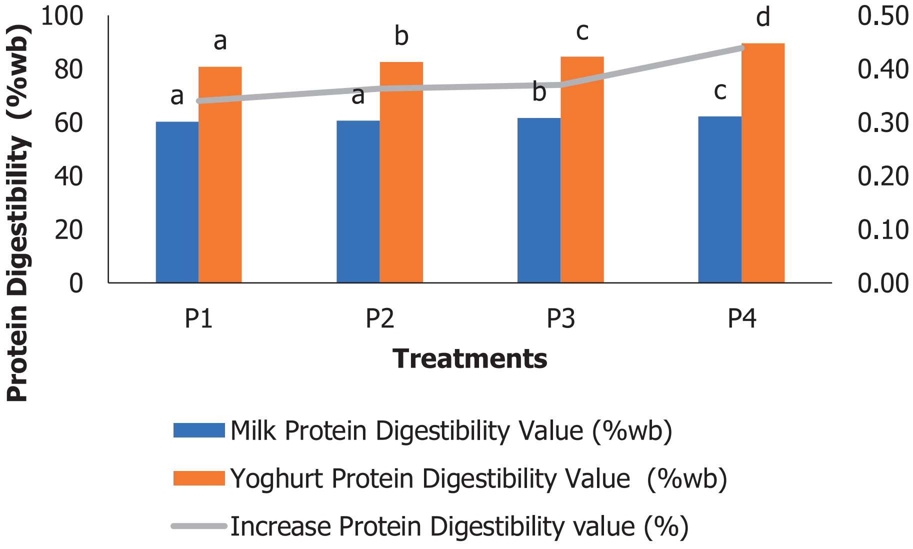

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Figure 3. Comparation of protein digestibility value in legume milk and legume yogurt

reaching 0.0025%. This compound has a strong chelating structure that forms insoluble complexes with divalent and trivalent mineral cations, including calcium, magnesium, zinc, copper, iron, and potassium(Irwan et al., 2024). Another anti-nutrient found in pigeon peas is tannins (Samben & Puspaningrum, 2020).

Mungbeans contain several anti-nutritional compounds that inhibit the absorption of nutrients, including phytic acid, tannins, lectins, and protease inhibitors (Winarsi et al., 2021). Meanwhile, antinutritional factors found in soybeans are protease inhibitors, lectins, phytoestrogens, oligosaccharides (stachyose and rafinose), phytic acid, and pectin (Hany El-Shemy, 2011). Among anti-nutritional factors in legume, protease inhibitors and tannins are the most directly associated with reduced protein digestibility (Gilani et al., 2012). Protease inhibitors decrease the activity of digestive enzymes such as trypsin and chymotrypsin, thereby limiting protein hydrolysis, while tannins can bind dietary protein and digestive enzymes to form insoluble complexes that reduce protein availability. In contrast, phytic acid primarily reduces mineral bioavailability through chelation and has a less direct effect on protein digestibility compared to protease inhibitors and tannins (Karlund et al., 2021).

The low digestibility of legume protein is due to the large multimeric globular-shaped framework that folds into an orderly and dense structure due to the presence of disulfide bonds, hydrophobic properties, electrostatic forces, hydrogen bonds, and Van der Waals forces (Goulding et al., 2019; Kieliszek et al., 2021). The hydrophobic nature of these proteins increases susceptibility to aggregation during denaturation, which further reduces protein digestibility (Harper et al., 2022). The appearance of antinutritional factors (ANF) such as lectins, saponins, polyphenols, and trypsin inhibitor affect protein digestion. In general, antinutritional components can decrease protein digestibility by inhibiting the accessibility and activity of digestive enzymes. For example, trypsin inhibitors found in legume are capable of reducing the binding to the active side of the enzyme. Polyphenols have also been shown to reduce protein digestibility by inactivating the activity of digestive enzymes due to the formation of polyphenol complex compounds (Santos-Sánchez et al., 2024).

Legume composition had a significant effect ($p<0.05$) on the protein digestibility of yogurt. Based on the results, yogurt from various types of legume were found to have protein digestibility values ranging from 80.86 to 89.60%wb. A single type of legume produced the lowest protein digestibility, while a combination of legume types caused significant improvement. The increase in yogurt protein digestibility is largely attributed to lactic acid fermentation, which can reduce anti-nutritional factors such as phytic acid, trypsin inhibitors, saponins, and tannins found in legume.

The activation of phytase and phosphatase enzymes due to a decrease in pH can degrade phytic acid, resulting in inorganic minerals and phosphates being available in free form without chelations. LAB degrades tannins through the activity of tanase and decarboxylates gallate. During the process, tannin is converted to tannic acid by tanase. Subsequently, tannic acid is hydrolyzed into gallic acid and glucose. The gallic acid formed is then decarboxylated into pyrogallic acid. The reduction of ANF contributes directly to improved protein digestibility (Montemurro et al., 2021). The increase in yogurt protein digestibility is positively associated with the rise in protein content resulting from the fermentation of LAB. The fermentation of milk in various types of legume not only changes the content and structure of protein but also increases the type and amount of amino acids, thereby enhancing the digestibility of protein and the nutritional value (Zhai et al., 2025).

Chemical Content

The chemical content of yogurt produced from different types of legume, including carbohydrate, fat, ash, and water content, is shown in Figure 4. According to SNI 2981:2009, the ash content of yogurt made from various legumes complies with the standard for animal-milk yogurt, which specifies a maximum ash content of less than 1%. However, fat content is less than 3%, indicating that it does not meet the minimum fat requirement specified in SNI 2981:2009. The level of carbohydrates, fats, and ash of yogurt produced from a single type of legume was low, while the combination of legume showed increased values for these parameters. However, the water content of yogurt was inversely proportional to the content of carbohydrates, fats, and ash.

The carbohydrate content in yogurt describes the amount of sugar (simple and complex), which comes from the activity of microorganisms. The legume type had a significant effect ($p < 0.05$) on the carbohydrate content of yogurt. The carbohydrate content of yogurt from various types of legume ranged from 9.52 to 14.96%. The variation in the carbohydrate content can be attributed to the different composition of the legume used. Pigeon peas are primarily composed of 0.14% glucose, 0.40% fructose, 4.02% sucrose, amylum, fiber, and oligosaccharides that cannot be directly utilized by LAB (Cirunay et al., 2021).

The carbohydrate profile in mungbeans in percentage include glucose (0.3), sucrose (1.3), Rafinosa (1.1), stachyose (1.6), verbascose (2.7), soluble dietary fiber (2.3), insoluble dietary fiber (15.3), amylose (24), starch (47), lignin (3.9), cellulose (3.9), and hemicellulose (4.7) (Dahiya et al., 2015). Soybeans similarly contain carbohydrates in the form of dietary fiber, sucrose, and raffinose-family oligosaccharides, such as raffinose, stachyose, and verbascose (Aanchal, 2023). Increased diversity and availability of fermentable carbohydrates for *L. bulgaricus* and *S. thermophilus* lead to higher carbohydrate content in yogurt (Alawiyah et al., 2024).

Fermentation by *L. bulgaricus* and *S. thermophilus* occurs through the conversion of carbohydrates into

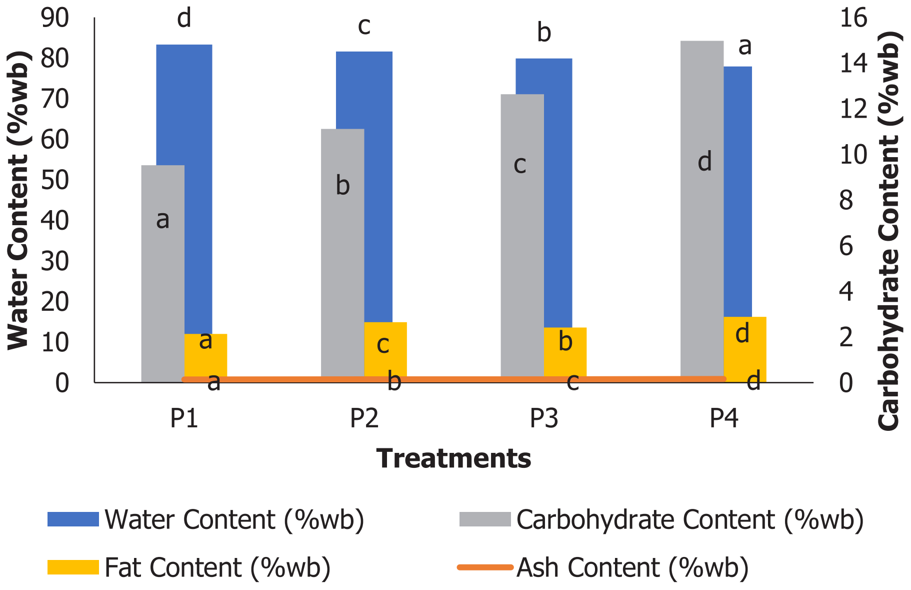

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Figure 4. Carbohydrates, fats, ash and water level of Legume Yogurt

glucose. Subsequently, glucose is fermented through the diphosphate hexose pathway to produce lactic acid as the main product (Layadi et al., 2009). Sucrose is hydrolyzed by sucrase (invertase) into glucose and fructose, which are subsequently metabolized through the Embden–Meyerhof pathway (glycolysis) to produce pyruvic acid. Under homofermentative conditions, pyruvate is mainly reduced to lactic acid by lactate dehydrogenase. Consequently, higher availability of fermentable carbohydrates supports increased lactic acid production during yogurt fermentation. Overall, carbohydrate content is directly proportional to lactic acid production, as LAB metabolize carbohydrate substrates through homofermentative pathways, with lactic acid as the primary metabolite (Fadhilla et al., 2024).

The composition of the legume type has a significant effect ($p < 0.05$) on the fat content of yogurt, which ranged from 2.14 to 2.88%. The increase in fat content after fermentation can be attributed to the biosynthesis pathway of esterolytic or lipolytic enzymes that play a role in fat degradation (Zhai et al., 2025). *L. bulgaricus* produces lipase to hydrolyze fats into fatty acids, and then fatty acids are broken down into compounds that have a characteristic yogurt aroma (Purwatiningsih et al., 2022). The fat content of yogurt is influenced by intrinsic factors of legume (the fat content of the ingredients and the structure of the cell wall) and extrinsic factors (activity during fermentation). Higher initial fat levels in legume milk can result in greater yogurt fat content, although fermentation by *L. bulgaricus and S. thermophilus* primarily induces limited lipolysis that contributes to flavor development rather than a substantial increase in total fat content. Therefore, the fat content of yogurt is largely determined by the fat concentration of the legume milk used.

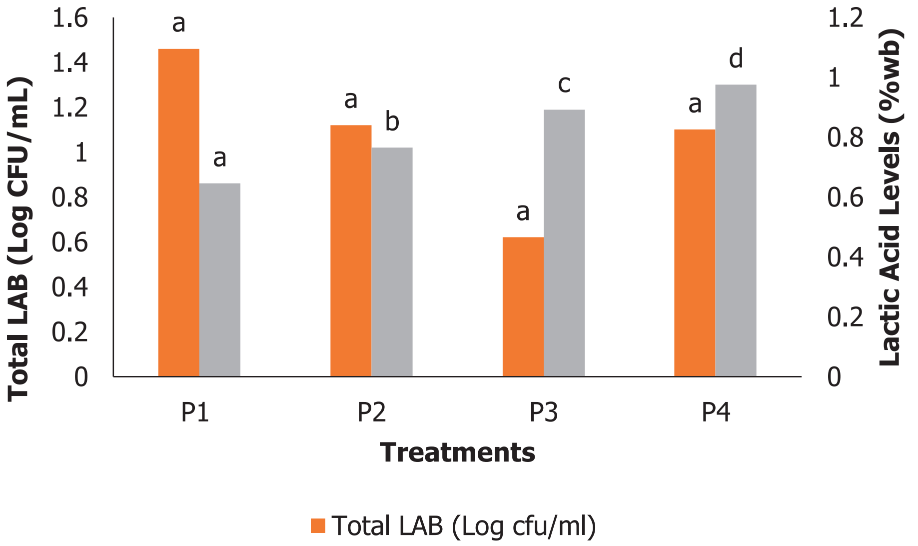
Among the legume used, pigeon peas had the lowest fat content of 1.2%, mungbeans contain about 9%, and the highest content was found in soybeans at 21% (Febriani et al., 2019; Gyeongseon et al., 2024). The lipid fraction of pigeon peas mainly consists of palmitic and linoleic acids (Haji et al., 2024). Mungbean lipids are composed of saturated fatty acids (27%), polyunsaturated fatty acids (44.61%), and monounsaturated fatty acids (28.39%) (Kumalasari & Fajriyati, 2024), while soybean lipids are dominated by linoleic acid.

These differences in fatty acid profiles influence the nutritional quality and oxidative stability of the yogurt. For example, higher proportions of unsaturated fatty acids may provide health benefits but can be more susceptible to oxidation, potentially affecting flavor and shelf-life. Although the total fat content mainly depends on the original fat level of the legume milk, fatty acid composition helps explain differences in product quality and stability among treatments. The low activity of the lipase enzyme in LAB for hydrolyzing unsaturated fatty acids increases yogurt fat content (Labiba et al., 2020). Fatty acid composition is important because it influences the nutritional value, oxidative stability, and sensory properties of yogurt, even though the total fat content is mainly determined by the initial fat concentration of the legume milk.

The ash content in yogurt describes the percentage of mineral content in the type of legume used. The legume type had a significant effect ($p<0.05$) on the ash content of yogurt, which ranged from 0.78 to 0.90%. Variations in ash content can be attributed to differences in both the type and concentration of minerals naturally present in each legume. Pigeon peas contain several types of minerals such as iron, sulfur, calcium, potassium, and manganese (Febriani dkk, 2019). Soybeans contain minerals such as calcium, iron, magnesium, and zinc (Zeinatulina et al., 2025), with reported concentration (g/kg) of calcium (2.62), phosphorus (5.70), magnesium (2.80), potassium (15.93), and sodium (0.29) (Hany El-Shemy, 2011). Meanwhile, the mineral composition of mungbeans (mg/100 g) includes calcium (113.4), copper (1.0), iron (5.9), potassium (956.6), magnesium (162.4), manganese (1.05), sodium (16.7), phosphate (384.4), and zinc (2.7) (Dahiya et al., 2015). The variation in yogurt ash content is mainly attributed to differences in the mineral composition of the legume used, because ash represents the total mineral fraction of the product.

The different mineral profiles among pigeon pea, soybean, and mungbean are expected to contribute to the observed differences in yogurt ash content. During fermentation, LAB, which are acid-tolerant, non-spore-forming microorganisms, can adapt to a wide range of food matrices, including milk, legumes, meat, fish, and vegetables. These bacteria obtain energy for growth through the metabolism and consumption of various compounds such as amino acids, fatty acids, simple sugars, and minerals (Emkani et al., 2022). The minerals contained in legume are released from chelation complex compounds through fermentation activities by *L. bulgaricus* and *S. thermophilus* to increase the ash content in yogurt (Lim et al., 2019).

The moisture content describes the amount of water contained in yogurt and affects the texture, quality, viscosity, and activity of the water (Aw). The legume type had a significant effect ($p < 0.05$) on the moisture content of yogurt, which ranged from 83.35 to 77.94 %. Reductions in moisture content during fermentation may be attributed to increased dry



Note: superscripts on bar charts with the same color indicated significant differences in the One-Way ANOVA test with significance level of 5% ($p < 0.05$). P1 = pigeon peas; P2 = pigeon peas + mungbeans; P3 = pigeon peas + soybeans; P4 = pigeon peas + soybeans+ mungbeans, n= 5 repetitions.

Figure 5. Total lactic acid bacteria and lactic acid level of Legume Yogurt

matter resulting from microbial biomass proliferation, water utilization during microbial metabolic activity, and an increase in total dissolved solids formed due to fermentation processes (Lim et al., 2019).

Total Lactic Acid Bacteria and Lactic Acid Content

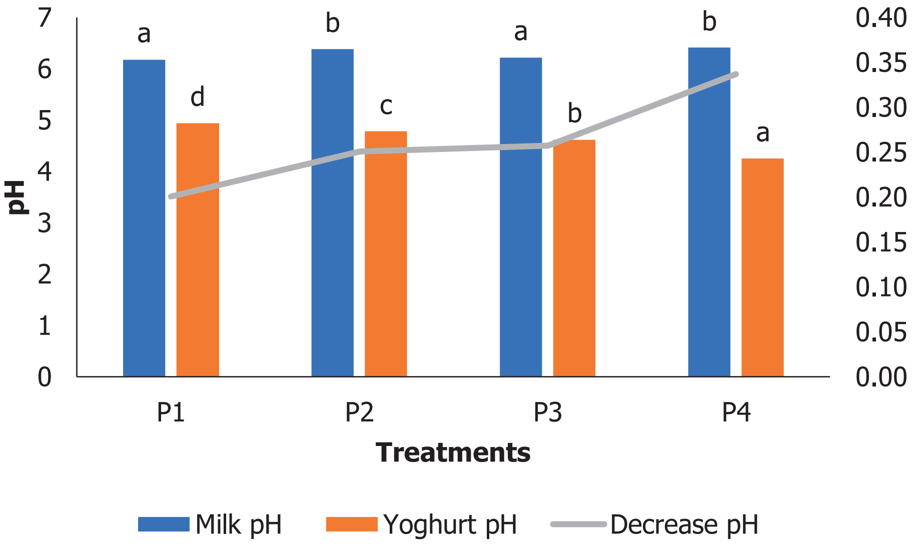
Total LAB and lactic acid levels of different types of legume yogurt are shown in Figure 5.

The legume type had no significant effect (sig>0.05) on total LAB of yogurt, which ranged from 4.16 $\log_9$ to 2.88 $\log_{10}$ CFU/mL. The lack of significant differences in LAB populations is possibly due to similarities in the nutritional composition of the legumes and the adaptability of LAB, which allows relatively uniform growth across all treatments. Pigeon peas, mungbeans, and soybeans provide nutrients that can serve as growth substrates for *Lactobacillus bulgaricus and Streptococcus thermophilus*. Key growth-promoting factors for LAB include oligosaccharides, amino acids, and peptides.

During fermentation, *L. bulgaricus* and *S. thermophillus* hydrolyze oligosaccharides such as rafinose and stachyose through α-galactose enzymes into sucrose and galactose, as well as metabolize protein, fats, and micronutrients (minerals and vitamins) (Elghali et al., 2014). Both *L. bulgaricus* and *S. thermophilus* generally show limited α-galactosidase activity, hence, raffinose and stachyose are not efficiently used by starter cultures and may only be partially metabolized (Elghali et al., 2014). All three types of legume have high nutrients (carbohydrates and fats) and a unique chemical composition that supports the proliferation of LAB (Jamalullail et al., 2023). In addition, the natural buffering capacity of legume promotes the survival of LAB (Parra et al., 2013).

Lactic acid in plant-based yogurt results from the fermentation of sugars, both disaccharides and oligosaccharides, by LAB. In contrast to total LAB counts, the type of legume significantly influenced ($p < 0.05$) lactic acid content, which ranged from 0.86% to 1.30%. Based on the data, fermentation using *L. bulgaricus* and *S. thermophilus* on a single type of legume produced the lowest lactic acid levels, while a combination of legume types increased lactic acid levels. Legume formulations have different abilities in stimulating the formation of lactic acid (Jamalullail et al., 2023). The lactic acid content observed in this study meets the SNI 2981:2009 standard for yogurt from animal milk (0.5%–2%).

The increase in lactic acid levels correlates with the nutrient levels of carbohydrates, protein, and fats in yogurt, indicating that these nutrients from pigeon peas, soybeans, and mungbeans are metabolized by *L. bulgaricus* and *S. thermophilus* into lactic acid. LAB are generally homofermentative, converting more than 85% of glucose or other hexoses into lactic acid (Nugroho et al., 2023). The more varied the source of legume, the greater the lactic acid in yogurt increases. This is due to the carbohydrate components of both monosaccharides (glucose, fructose), disaccharides (sucrose), and



Note: superscripts on bar charts with the same color indicated significant differences in the One-Way ANOVA test with significance level of 5% ($p < 0.05$). P1 = pigeon peas; P2 = pigeon peas + mungbeans; P3 = pigeon peas + soybeans; P4 = pigeon peas + soybeans+ mungbeans, n= 5 repetitions.

Figure 6. Comparation of pH in legume milk and legume yogurt

oligosaccharides found in pigeon peas, soybeans, and mungbeans, which can be used as a source of carbon for *L. bulgaricus* and *S. thermophilus.*

During the fermentation process, LAB hydrolyzes carbohydrate sources through the activity of α and β galactosidase into simple sugars and produces short-chain fatty acids, especially acetic acid, lactate, propionate, butyrate, along with gases, namely CO_2, CH_4, and H_2 (Elghali et al., 2014; Gyeongseon et al., 2024). *L. bulgaricus* and *L. acidophilus* use the resulting reduced sugars as an energy source. Lactic acid concentration is also associated with acid buffer capacity, which is positively affected by dissolved solids content and protein content, especially high glutamic and aspartic acid content (Jamalullail et al., 2023).

pH

pH is defined as the acidity level of a food product, and the pH content of legume milk and yogurt is shown in Figure 6.

Figure 6 shows that the legume type had a significant effect ($p < 0.05$) on the pH level of milk. The pH of the milk from different types of legume ranged from 6.18 to 6.41. Based on the data, the pH value of pigeon peas milk, as well as the combination of pigeon peas milk + mungbeans, was lower (acidic) compared to pigeon peas milk + soy and pigeon peas milk + soy + mungbeans. This variation is possibly due to differences in the nutritional composition of the legumes and the presence of similar anti-nutrient compounds, including phytates, tannins, and saponins, which exhibit acidic properties. (Lim et al., 2019). Additionally, carbohydrate composition, types and levels of amino acids (e.g., glutamic acid), phenolic compounds, and mineral content influence the pH of legume-based milk.

The legume type had a significant effect ($p<0.05$) on the pH level of yogurt, which ranged from 4.25 to 4.94. Based on the data, there was a decrease in pH from legume milk to yogurt. This implies that pigeon peas, mungbeans, and soybeans have the potential to support LAB growth without animal milk supplementation, as evidenced by pH value decrease during fermentation. *Plant-based yogurt* must have a pH of 4.6 to 4.9 and be fermented at a temperature of 36 °C – 45 °C for 6-18 hours (Dhakal et al., 2023). LAB activity lowers pH by converting carbohydrates, hexoses, oligosaccharides, and complex sugars into lactic acid (Jamalullail et al., 2023). Consequently, higher lactic acid production correlates with lower pH values, as observed in this study (Jamalullail *et al.*, 2023).

L. bulgaricus and S. thermophilus are heterofermentative bacteria that produce α-glucosidase, an enzyme capable of hydrolyzing α-1,6-glycosidic bonds in carbohydrates to generate lactic acid (Cirunay et al., 2021). The process of glucose catabolism causes *L. bulgaricus* to synthesize pyruvic acid, which stimulates the growth of *S. thermophilus,* thereby increasing the acidity level (Nugroho et al., 2023). The type of starter also affects the pH value of yogurt. The combination of *S. thermophilus* and *L. bulgaricus* in the fermentation of vegetable milk produced yogurt with a good pH and taste. This is because *S. thermophilus* facilitates the formation of sour taste and stimulates the growth of *L. bulgaricus*, which in turn produces volatile compounds and lactic acid capable of affecting the aroma of yogurt (Kumalasari & Fajriyati, 2024).

CONCLUSION

In conclusion, the legume type has a significant effect on proximate levels, dissolved protein, protein digestibility, lactic acid, and pH, but not on total LAB of yogurt. Based on the results, yogurt produced from a single type of legume had lower proximate levels, dissolved protein, protein digestibility, lactic acid, and pH. Meanwhile, the combination of two or three types of legume increased the proximate levels, dissolved protein, protein digestibility, and lactic acid. Pigeon peas+soybeans yogurt yielded higher proximate levels, dissolved protein, protein digestibility, and lactic acid but had lower pH than pigeon peas+mungbeans yogurt. Pigeon peas+soybeans+mungbeans yogurt produced the highest proximate, dissolved protein, protein digestibility, and lactic acid content. Therefore, combining pigeon peas, soybeans, and mungbeans milk improved protein digestibility and nutritional profile without affecting LAB viability, suggesting the potential as a functional dairy alternative.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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