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Antibacterial Activity of Bioactive Peptides Kacang Goat Milk and African Dwarf Breed Milk Fermented with *Lactobacillus acidophilus* and *Bifidobacterium longum*

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ABSTRACT. This study evaluated the antibacterial activity of bioactive peptides derived from African Dwarf crossbreed and Kacang goat milk fermented with *Lactobacillus acidophilus* and *Bifidobacterium longum*. Bioactive peptide fractions were obtained using Sephadex G-25 column separation, and their antibacterial activity was assessed using the well diffusion assay. Inhibition zones were measured at different fermentation times to compare the activity profiles between the two probiotic strains. Fermentation with *Lactobacillus acidophilus* and *Bifidobacterium longum* produced bacteriostatic effects, with inhibition zones ranging from 1.10 mm to 1.77 mm and activity persisting up to 24–36 hours, depending on strain and fermentation condition. These findings show that goat milk fermented for 12–18 hours is capable of producing bacteriocin-rich peptide fractions with antibacterial potential.

Keywords: Antibacterial, *Bifidobacterium longum*, Bioactive peptides, Goat milk, *Lactobacillus acidophilus*.

INTRODUCTION

Dairy goat breeds that are often kept by dairy goat breeders include the Peranakan Ettawa, Bligon, Saanen, and Sapera. (Suranindyah et al., 2018). Goat milk is recognized for its favorable nutritional profile, digestibility, and bioactive components, making it an increasingly relevant source of functional foods. The composition of goat milk varies among breeds, influenced by genetic and environmental factors. Kacang goats represent an indigenous Indonesian breed characterized by moderate milk yield, while African Dwarf goats, recently introduced and adapted to tropical climates, exhibit higher milk productivity. Despite their different origins, these breeds have not been extensively compared in terms of the functional properties of their milk, particularly regarding the potential to generate bioactive peptides during fermentation. Goat's milk has an average fat content of 3.9 to 5.7%, protein content of 3.85%, water content of 86.49%, and total solids of 4.59% (Murti, 2016). The quality and composition of goat's milk are similar to human milk, and it even contains higher levels of calcium and other minerals than either human milk or cow's milk (Rusdiana, et. al. 2016). This research is a preliminary step in determining whether breed influences the growth of lactic acid bacteria (LAB), which carries functional traits, particularly the genetic differences between local goats and imported African goats.

Milk fermentation by LAB facilitates the proteolysis of casein and whey proteins into bioactive peptides with antimicrobial properties. The fermentation process improves the nutritional quality of milk; besides that, it can also increase the antibacterial activity of goat milk. *Lactobacillus acidophilus* and *Bifidobacterium longum* are widely recognized probiotic strains capable of producing bacteriocins such as acidophilin and bifidobactin, respectively. Small bacteriocins (less than 6500 Da) produced by *L. acidophilus*, called acidophilin, have potential probiotic properties (Zamfir et al., 2000). These low molecular weight peptides exhibit bacteriostatic effects against competing microbial populations. *B. longum* can naturally produce an antibiotic called bifidobactin (Hanum et al., 2022). Differences in primary and secondary milk protein sequences between goat breeds may influence peptide release during fermentation, suggesting that the antibacterial activity of fermented milk may vary according to breed-specific protein composition.

However, comparative studies evaluating the antibacterial activity of fermented milk peptides derived from different goat breeds remain limited. Therefore, this study aimed to evaluate the antibacterial activity of bioactive peptide fractions produced through the fermentation of Kacang and African Dwarf goat milk using *L. acidophilus* and *B. longum*. The inhibitory effects were assessed against selected bacterial indicators to determine whether breed-specific protein characteristics affect the antimicrobial potential of the resulting peptides.

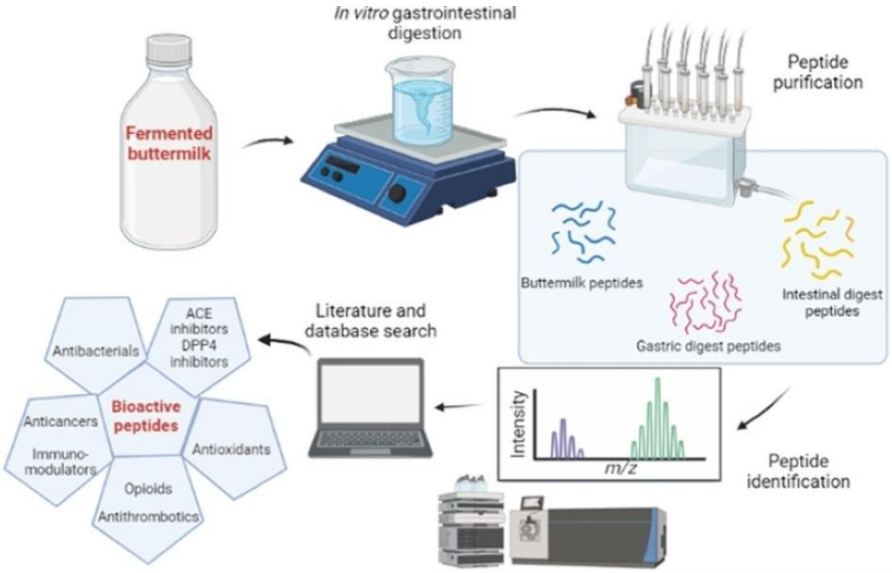

Figure 1. Bioactive peptides detection (Magouz et al., 2023)

EXPERIMENTAL SECTION

Material

Milk samples were obtained from Indolait Farm, Sleman, Yogyakarta (Kacang and African Dwarf breeds). A total of 2 L of each milk type was used. A Bacteria's isolate culture were obtained from Pusat Studi Pangan dan Gizi, Gadjah Mada University.

Methods

Fermentation was conducted separately for each treatment batch, with three replications for each treatment.

Determination of Total Solids

The porcelain crucible was heated in an oven at 105°C for one hour. After cooling in a desiccator for 30 minutes, the empty crucible was weighed. Approximately 1 g of milk sample was placed in the crucible and dried in an oven at 105°C for a minimum of six hours. The crucible containing the dried sample was cooled in a desiccator for 30 minutes and then weighed to determine total solids.

Determination of pH and Acidity Levels

pH was measured using a digital pH meter by immersing the electrode in the milk sample (SNI 1992). Titratable acidity was determined using the Mann-Whitney titration method (Hadiwiyoto, 1982). Nine milliliters of milk sample were placed in an Erlenmeyer flask, three drops of phenolphthalein indicator were added, and the sample was titrated with 0.1 N NaOH until a persistent pink endpoint was reached. Acidity was calculated following Hadiwiyoto (1982). Measurements were performed on fresh milk and repeated after each incubation time.

Determination of Protein Levels

The milk sample was weighed at around 1 gram and then placed in a Kjedahl flask, and then 7.5 grams of $K_2S_2O_4$ and 15 mL of concentrated H_2SO_4 were added. After that, the tube containing the sample is heated over an electric heater (destruction process) in an acid cupboard until it boils and the solution is clear greenish in color (around 2 hours), then diluted using distilled water into a volumetric flask to a volume of 100 mL up to the line mark. The resulting digestion solution was put into a distiller as much as 5 mL with 5 mL of 30% NaOH added and about three drops of pp indicator, then distilled using a distillation apparatus for 10 minutes. The steam resulting from the distillation was collected into an Erlenmeyer flask, which had been filled with 10 mL of 2% boric acid (H_3BO_3) mixed with a mix indicators, to a volume of 75 mL. Then titration was carried out using 0.01 N HCl (SNI, 1992).

Determination of Lactose Levels

The fermented milk lactose content test was carried out using the method. (Sudarmadji et al., 1997). 25 mL milk sample was put into a 50 mL volumetric flask and 5 mL of $ZnSO_4$ reagent then shaken. Then 5 mL of NaOH was added (93 g of NaOH diluted to 3 liters = 0.75 N), then shaken, then diluted until the lines were marked on the flask. The suspension containing the sample was left for 10 minutes to precipitate the protein, then filtered with filter paper, and then the filtrate and the volume were calculated. 5 mL of the clear filtrate was taken and then put into a 250 mL Erlenmeyer flask. Add 20 mL of distilled water and 20 mL of 10% KI solution, then add 50 mL of Chloramine-T solution and cover the flask with plastic, then shake gently. The solution was left for 90 minutes, then 10 mL of 2 N HCl solution. Next, it was titrated using a 0.1 N $Na_2S_2O_3$ solution until it changed to a pale yellow color. Next, add the starch solution and titrate again until it turns gray. Make a blank solution by replacing 25 mL of the sample with 25 mL of distilled water.

Goat Milk Fermentation

Kacang and African Dwarf goat milk samples (2 L

each) were obtained from Peternakan Indolait, Sleman. Each milk type was heated in sterile Schott bottles (1,000 mL) at 80°C for 30 minutes, then cooled to 40°C. The milk from each container was divided into 20 sterile bottles (100 mL each) and inoculated with either *L. acidophilus* or *B. longum* at 5% (v/v) of milk volume. Additional nutrients (0.045 g glucosamine for *L. acidophilus* and 0.045 g galactosamine for *B. longum*) were added. Fermentation was performed separately for each treatment and incubated at 38°C for 0, 6, 12, 18, and 24 hours (three replicates per treatment).

Protein Extraction Using Sephadex G-25

Protein extraction followed Herlina et al. (2019) with modifications. Sephadex G-25 gel was equilibrated in 25 mM Tris-HCl buffer pH 5.5 and packed into a 15 mL chromatography column. Fermented milk samples (0, 12, 18, 24 h) were centrifuged (4,500 rpm, 4°C, 20 min), and the supernatant was precipitated with 80% ammonium sulfate at 4°C. The precipitated supernatant was applied to the Sephadex G-25 column and eluted with 25 mM Tris-HCl (pH 5). Eluates were visually separated by color into fractions corresponding to approximate molecular weight ranges: clear (MW > 5.0 kDa), yellowish (MW 1.5–5.0 kDa), and bright yellow (MW < 1.5 kDa). The yellowish fraction (1.5–5.0 kDa) was collected for antibacterial testing.

Antimicrobial Activity Assay

This method is a modification of (Sharma et al., 2018), MRS agar was prepared and autoclaved following manufacturer instructions. Plates were inoculated with the test organism (rejuvenated cultures diluted to 10^-7) mixed into molten agar. Wells (10 mm diameter) were made in solidified agar and filled with Sephadex G-25 extracts from corresponding fermentation treatments. Plates were incubated at 38°C and inhibition zone diameters were measured every 12 hours up to 72 hours.

Data Analysis

Data for milk composition and antibacterial assays were analyzed using independent sample t-tests where appropriate. The experimental design was a split-plot with two factors: goat breed (Kacang, African Dwarf) and bacterial strain (*L. acidophilus*, *B. longum*), each with two levels and three replicates per treatment. Significant differences were reported at $p < 0.05$.

RESULTS AND DISCUSSION

Composition of Fresh Milk

The results of testing the composition of Kacang and African goat milk were presented in **Table 1**. Kacang goat milk has a higher total solid than African goat milk; both are not significantly different. Differences in total solid levels can be caused by genetic polimorfism, nutritional feed consumption, animal age, and milk production. The pH value of Kacang goat milk is not significantly higher than African goat milk, but both still comply with the 2011 SNI milk quality standard, milk pH between 6.3 to 6.8. The results of protein testing showed that the crude protein content in African goat milk was not significantly higher than in Kacang goat milk. This protein level indicates the number of amino acids contained in goat milk, and there are bioactive peptide components in it. The lactose content of Kacang goat milk is lower than African goat milk, but not significantly different.

Composition of Fermented Milk

Total solid value ingredients of Kacang and African goat milk fermented using *L. acidophilus* and *B. longum* bacteria are presented in **Figure 2**. Fermented Kacang goat milk has higher total solid than fermented African goat milk. Total solid value of Kacang and African goat milk fermented using *L. acidophilus* and *B. longum* in this study decreased with increasing fermentation time. The longer the incubation time, the thicker the fermented milk becomes due to proteins that can coagulate in an acidic atmosphere. The decrease in the total solid of fermented milk is related to a decrease in the ability of milk proteins to bind water due to hydrolysis by the activity of probiotic bacteria. This causes the water contained in the protein elements to dissolve together with other amino acid components, so that the water content tends to increase. The decrease in total solid levels is caused by lactose levels decreasing due to metabolic processes in LAB. The acidity levels of Kacang goat milk and African goat milk fermented with *L. acidophilus* and *B. longum* can be seen in **Figure 3**.

pH value of milkgoat African, and Kacang goat milk fermented with *L. acidophilus* and *B. longum* can be seen in **Figure 4**. Fermentation time has a very significant ($p<0.01$) effect on the acidity and pH value of Kacang and African goat milk, whether fermented with *L. acidophilus* or *B. longum*. The longer the fermentation, the pH value decreases while the acidity level increases. The difference in pH and acidity levels in the 12th to 18th hours of fermentation shows that secondary metabolites, namely bacteriocins, or bioactive peptides, begin to be produced at that hour. Lactic acid bacteria began to produce secondary metabolites, including bacteriocins, at 6 hours of incubation, marked by a smaller decrease in pH. Bacteriocins are ideally produced at pH conditions between 5.0 to 6.0, so that when the pH approaches 4 at the 18th hour, the decrease value is smaller. With the activity of lactic acid bacteria, the lactose levels in yogurt will decrease, and there will be an increase in lactic acid levels, which affects the acidity of the yogurt.(Agustina, et al., 2015).

The protein content of Kacang goat milk and African goat milk fermented with *L. acidophilus* and *B. longum* can be seen in **Figure 5**. Fermentation time has a significant effect ($p<0.01$) on the protein content

of fermented milk, which is related to the proteolytic activity of lactic acid bacteria. The metabolism of lactic acid bacteria will produce metabolites in the form of protease enzymes, which can hydrolyze proteins into peptides, dipeptides, tripeptides, and oligopeptides. Next, the peptides will be transferred into the lactic acid bacteria cells and produce free amino acids. These free amino acids increase the protein content test. Nitrogen resulting from lactic acid bacteria metabolites becomes more easily released, so that it can be maximally quantified in the titration process, so that protein levels are higher (Rizqiati et al., 2021).

Table 1. Composition of Kacang and African goat milk

Parameter	Kacang goat milk	African goat milk	P Value
Total solid (%)	15.18±0.30	14.32 ± 0.21	0.585 ns
Acidity(%)	0.17±0.01	0.19±0.02	1.000 ns
pH	6.68 ± 0.01	6.51±0.01	0.653 ns
Protein(%)	4.18 ± 0.16	4.28 ± 0.21	0.726 ns
Lactose(%)	4.09 ± 0.27	4.18±0.23	0.780 ns

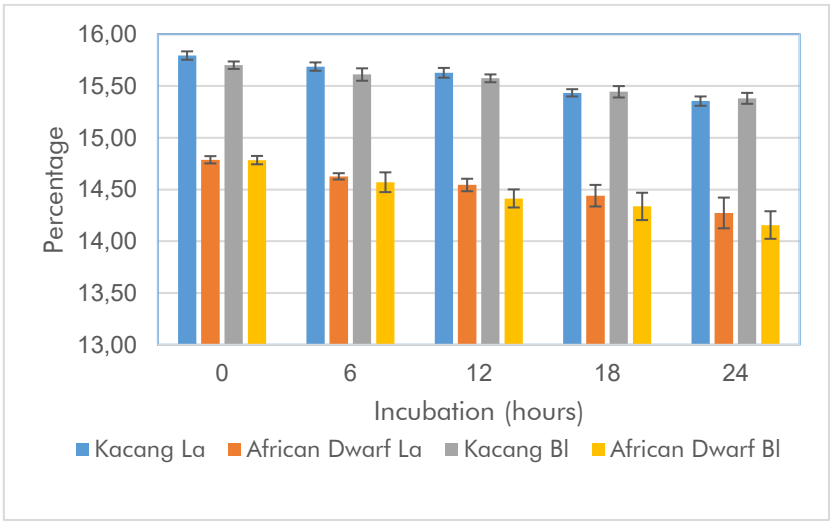

Figure 2. Total solid content (%) of fermented Kacang and African goat milk

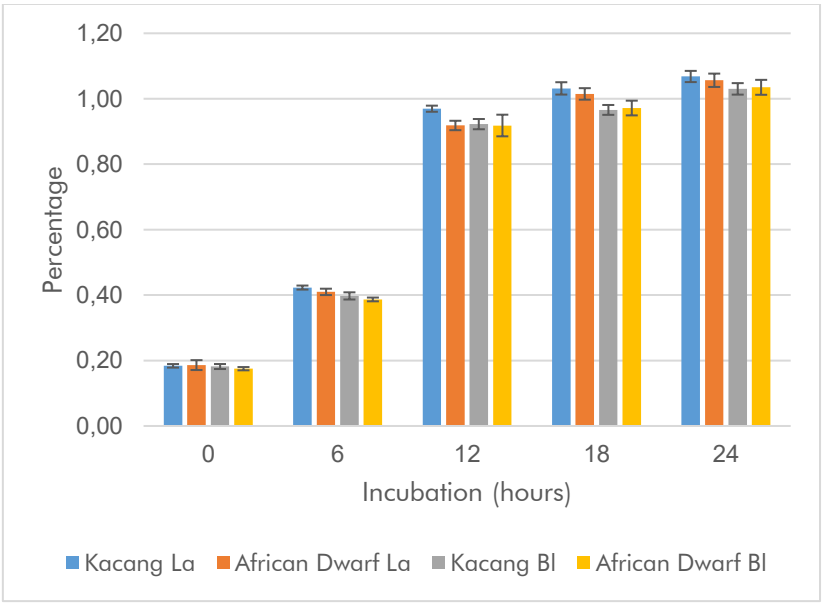

Figure 3. Acidity content (%) of fermented Kacang and African goat milk. Acidity level of fermented goat milk P<0.01.

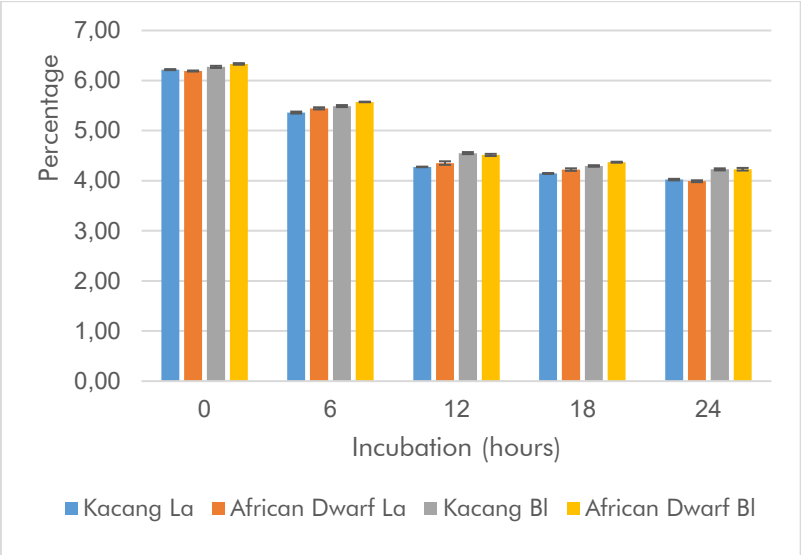

Figure 4. pH Value of Fermented Kacang and African goat milk. pH in fermented goat milk $P<0.01$.

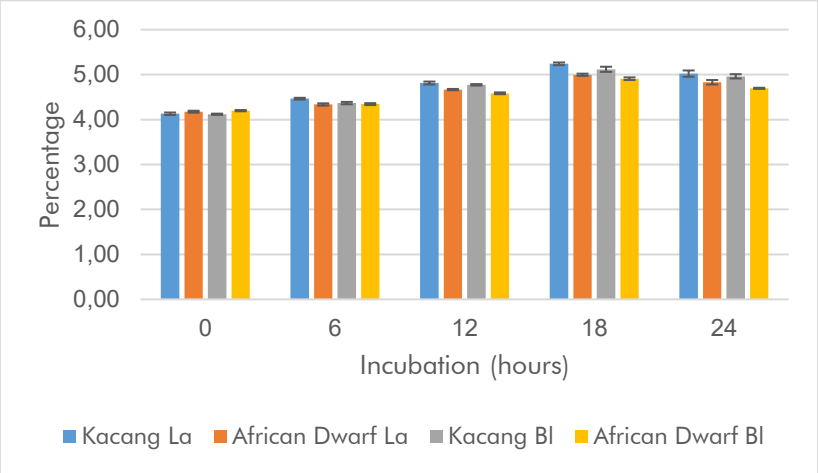

Figure 5. Protein content (%) of fermented Kacang and African goat milk. Protein content in fermented goat milk $P<0.05$.

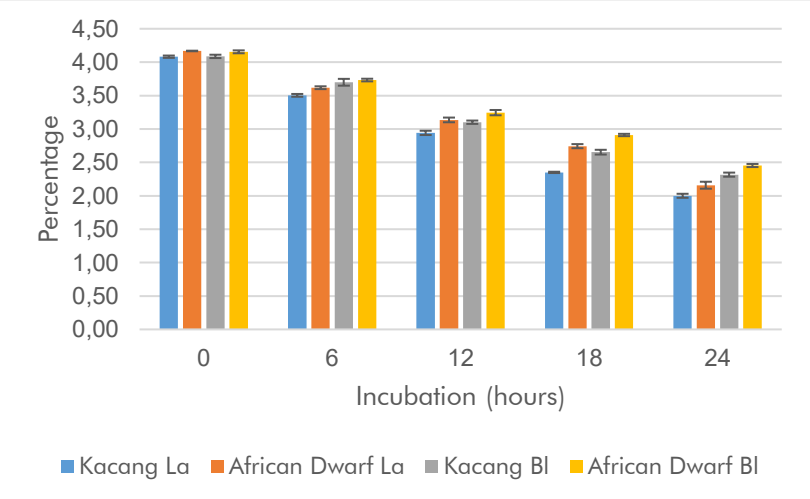

Figure 6. Lactose content (%) of fermented kacang and African goat milk. Lactose level in fermented goat milk $P<0.01$

The increase in protein begins to occur from the 6th to the 18th hour, which indicates the beginning of secondary metabolism, which produces bioactive peptides in the form of bacteriocins. Bacteriocins are proteins produced by LAB as a result of polypeptide metabolites. Bacteriocins began to form after 8 hours of incubation and reached optimal production within 18 hours. Protein levels decreased after 18 hours of fermentation. (Mokoena, 2017).

The lactose content of Kacang and African goat milk fermented with *L. acidophilus* and *B. longum* can be seen in **Figure 6**. Fermentation time has a very significant effect ($p<0.01$) on the lactose content of fermented milk; this is related to the metabolism of lactic acid bacteria. Lactic acid bacteria use the lactose in milk as an energy source for growth and reproduction. Milk lactose will be hydrolyzed using the lactase enzyme into glucose and galactose (Rizqiati et al., 2021). The process of fermenting lactose into lactic acid is that lactic acid bacteria use glucose in the lactose group of milk as a carbon source to produce pyruvate through glycolysis, then produce lactic acid with the help of the enzyme lactate dehydrogenase. Lactic acid is the final product in the metabolism of homofermentative lactic acid bacteria.

Goat milk fermented using *L. acidophilus* and *B. longum* for 24 hours still contained a small amount of lactose, indicating that the bacteria used did not use all the lactose in the milk. This is because the bacteria used in the milk fermentation process first utilize glucosamine and galactosamine, which are added at the beginning of incubation. LAB utilizes simpler energy sources first than breaking down lactose.

Antibacterial Activity of Kacang Goat Milk using the Well Method

Antibacterial activity in the extraction of Sephadex G-25 from Kacang goat milk fermented with *L. acidophilus* on MRSA containing *L. acidophilus* can be seen in **Figure 7**. Determination of antibacterial properties in research is based on differences in the molecular weight of fermented milk peptide proteins. Peptides with molecules larger than 1.5 KDa first flowed through the Sephadex G-25 gel. In this study, protein extracts from fermented milk with molecular weights between 1.5 KDa and 5.0 KDa were used.

Figure 7 shows the presence of antibacterial activity in the form of inhibiting the growth of *L. acidophilus* by the extraction of Sephadex G-25 from goat milk fermented with *Lactobacillus acidophilus*. The highest inhibitory activity occurred in goat milk fermented for 12 hours at 0.9 mm and lasted up to 36 hours of incubation. Afterwards, LAB can adapt to the same bacteria, thus forming a colony of *L. acidophilus* bacteria. Antibacterial activity in the extraction of Sephadex G-25 from Kacang goat milk fermented using *B. longum* on MRSA containing *B. longum* can be seen in **Figure 8**.

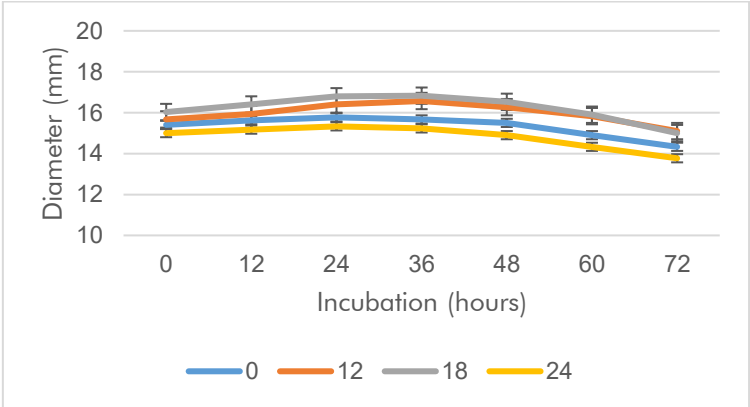

Figure 7. Inhibition zone fermented Kacang goat milk with *L. acidophilus* in a medium *L. acidophilus*. Inhibition zone fermented Kacang goat milk with *L. acidophilus* in a medium *L. acidophilus* ($P<0.05$)

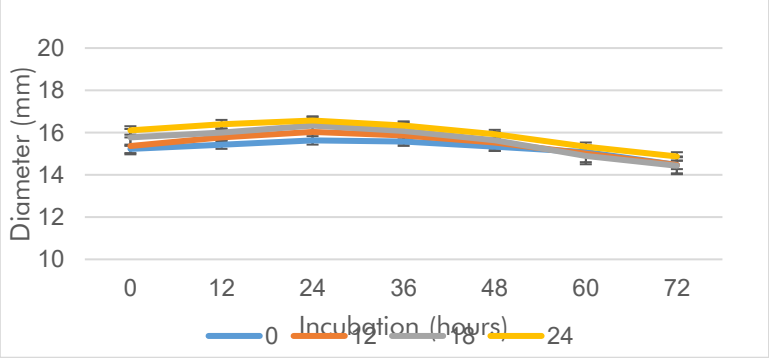

Figure 8. Inhibition zone fermented Kacang goat milk with *B. longum* in a medium *B. longum*. Inhibition zone fermented Kacang goat milk with *B. longum* in a medium *B. Longum* ($P<0.05$)

Figure 8 shows the presence of antibacterial activity in the form of inhibiting the growth of *B. longum* bacteria by extracting Sephadex G-25 from Kacang goat milk fermented with *B. longum*. The highest inhibitory activity occurred in goat milk fermented for 12 hours, with a diameter of 0.67 mm, but it only lasted up to 24 hours of incubation. Afterwards, LAB can adapt to the same bacteria and can grow.

The antibacterial activity of the extraction of Sephadex G-25 from African goat milk fermented with *L. acidophilus* on MRSA containing *L. acidophilus* can be seen in **Figure 9**. **Figure 9** shows that there is antibacterial activity in the form of inhibiting the growth of *L. acidophilus* by extracting Sephadex G-25 from African goat milk fermented by *L. acidophilus*. The highest inhibitory activity occurred in goat milk fermented for 12 hours at 0.87 mm and lasted up to 36 hours of incubation. These results are not much different from Kacang goat milk fermented using *L. acidophilus*, in that bacteriocins are bacteriostatic, or inhibit the growth of bacteria in the media without killing them.

The antibacterial activity of the extraction of Sephadex G-25 from African goat milk fermented using *B. longum* on agar media containing *B. longum* can be seen in **Figure 10**. **Figure 10** shows the antibacterial activity in the form of inhibiting the growth of *B. longum* bacteria by extracting Sephadex G-25 from African goat milk fermented by *B. longum*. The highest inhibitory activity occurred in goat milk fermented for 18 hours, with a diameter of 0.93 mm, and it was able to survive up to 36 hours of incubation. After that, the diameter of the wells decreased, indicating a back reaction by LAB on the MRSA.

The results of antibacterial testing of fermented Kacang goat milk and African with *L. acidophilus* and *B. longum* bacteria using the well diffusion method with the same type of bacteria showed bacteriostatic antibacterial activity for up to 36 hours. The diameter of the well stopped widening and moved towards the center of the well after 36 hours of incubation. This is thought to have occurred because there was a back reaction by the bacteria contained in the MRSA medium. It is thought that the bacteria in fermented milk die because the nutrients in the milk have been used up, so that bacteriocin activity stops and stimulates the growth of probiotic bacteria in the MRS Agar medium. Javvadi et al., (2022) Stated that host isolates or genetic variants of similar bacteria are often resistant to the original bacteriocin, causing lysis. The addition of probiotics increases the antibacterial properties of the yogurt. (Çakmakoğlu et al., 2024).

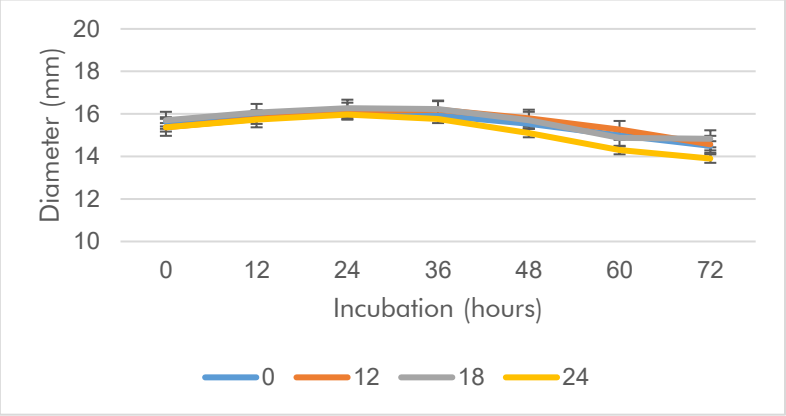

Figure 9. Inhibition zone fermented African goat milk with *L. acidophilus* in a medium *L. acidophilus.* Inhibition zone fermented African goat milk with *L. acidophilus* in a medium *L. acidophilus* (P<0.05)

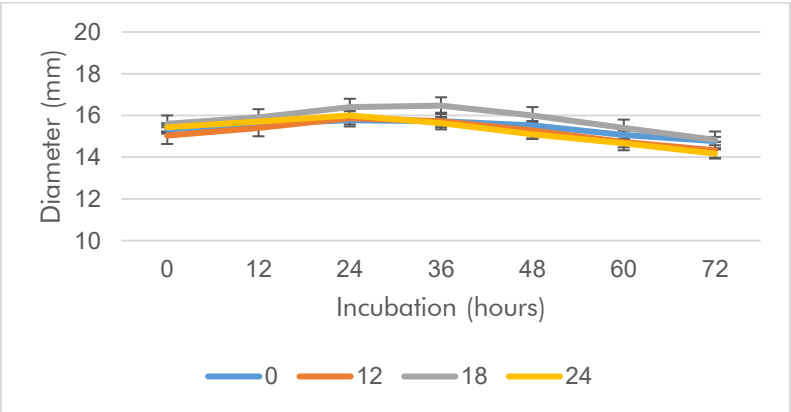

Figure 10. Inhibition zone fermented African goat milk with *B. longum* in a medium *B. longum.* Inhibition zone fermented African goat milk with *B. longum* in a medium *B. longum* (P<0.05)

Antibacterial activity in the extraction of Sephadex G-25 from Kacang goat milk fermented with *L. acidophilus* on agar media containing *B. longum* can be seen in **Figure 11**. **Figure 11** shows the presence of antibacterial activity in the form of inhibiting the growth of *B. longum* by extracting Sephadex G-25 from Kacang goat milk fermented by *L. acidophilus*. The highest inhibitory activity occurred in goat milk fermented for 12 hours, with a diameter of 1.77 mm, and lasted up to 36 hours of incubation. After 36 hours, the *B. longum* bacteria were able to grow and adapt to the *L. acidophilus* bacteria, thus forming a commensal symbiosis. The bacteria contained in fermented milk continue to grow and produce bacteriocins for up to 36 hours. Bacteria around the wells are suppressed by the bacteriocins produced by LAB in fermented milk.

Antibacterial activity in the extraction of Sephadex G-25 from Kacang goat milk fermented with *B. longum* on agar media containing *L. acidophilus* can be seen in **Figure 12**. The results in **Figure 12** show that there is antibacterial activity in the form of inhibiting the growth of *L. acidophilus* bacteria by extracting Sephadex G-25 from goat milk fermented by *B. longum*. The highest inhibitory activity occurred in goat milk fermented for 18 hours, with a diameter of 1.53 mm, and lasted up to 36 hours of incubation. This result is different from Kacang goat milk, which was fermented using *L. acidophilus* and tested on media containing *B. longum* (**Figure 11**), showing that the highest bacteriostatic activity occurred in milk fermented for 12 hours.

The inhibitory activity of *B. longum* tends to be lower than *L. acidophilus*. It is suspected that *L. acidophilus* has relatively faster growth, and milk fermented using *B. longum* produces metabolites that provide additional nutrition for *L. acidophilus*. The metabolism of *B. longum* produces organic acids; apart from that, it also produces B vitamins and exopolysaccharides (Yu et al., 2023).

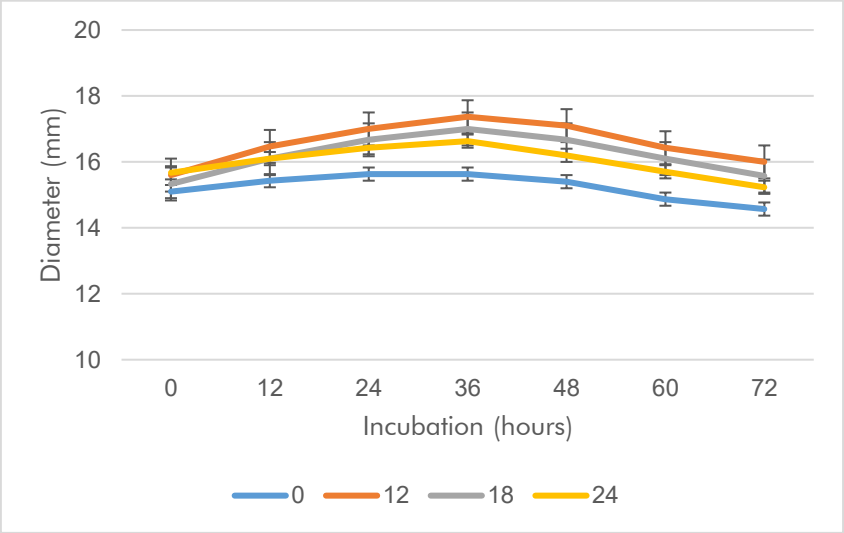

Figure 11. Inhibition zone fermented Kacang goat milk with *L. acidophilus* in a medium *B. longum*. Inhibition zone fermented Kacang goat milk with *L. acidophilus* in a medium *B. longum* ($P<0.05$)

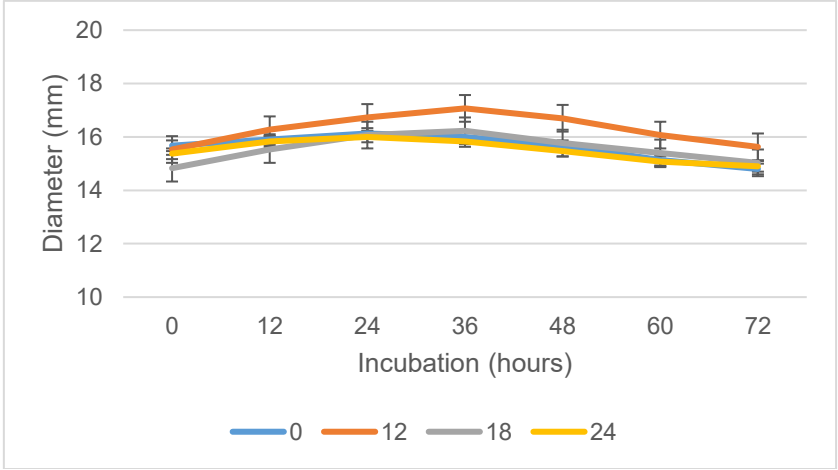

Figure 12. Inhibition zone fermented Kacang goat milk with *B. longum* in a medium *L. acidophilus*. Inhibition zone fermented Kacang goat milk with *B. longum* in a medium *L. acidophilus* ($P<0.05$).

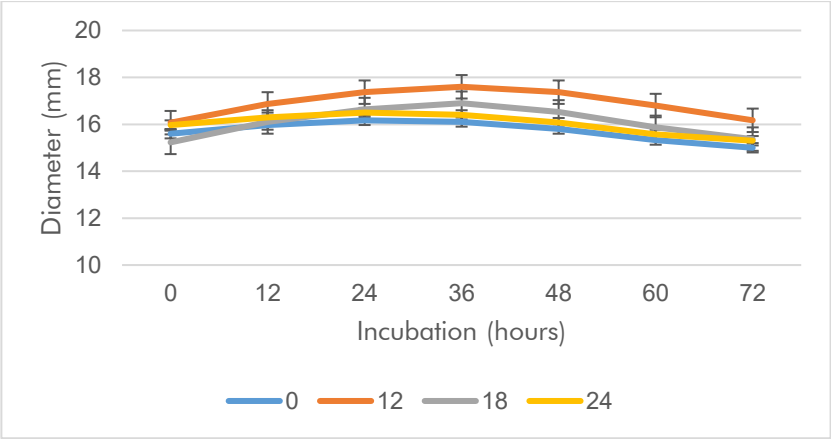

Figure 13. Inhibition zone fermented African goat milk with *L. acidophilus* in a medium *B. longum.* Inhibition zone fermented African goat milk with *L. acidophilus* in a medium *B. longum* (P<0.05).

The antibacterial activity of the extraction of Sephadex G-25 from African goat milk fermented with *L. acidophilus* on agar media containing *B. longum* can be seen in **Figure 13**. Based on the results of the antibacterial test in **Figure 13**, there is antibacterial activity in the form of inhibiting the growth of *B. longum* by extracting Sephadex G-25 from African goat milk fermented by *L. acidophilus*. The highest inhibitory activity occurred in goat milk fermented for 18 hours at 1.67 mm and lasted up to 36 hours of incubation. These results tend to be insignificantly different from Kacang goat milk fermented using *L. acidophilus* and tested on media containing *B. longum* (**Figure 11**); however, the highest bacteriostatic activity occurred in goat milk fermented for 18 hours. It is suspected that the bioactive peptides of *B. longum* bacteriocin were highest when incubated in milk for 18 hours.

Fermentation using probiotics activates amino acid peptides in milk. Until now, peptides with different antimicrobial activity have been detected in milk, of which lactoferrin is one of the most important ones. Enzymatic digestion of lactoferrin leads to the production of other antimicrobial peptides with more killing or inhibitory activity than lactoferrin (Besharati & Lackner, 2023).

(Zanutto-elgui et al., 2019) stated that the peptide sequence of bovine milk α-s2-casein FALPQYLK f(188–196) exhibits strong antimicrobial activity. The results are similar to previously described sequences, as well as two other similar sequences found in bioactive peptides from goat milk, namely ISQYYQK f(182–189) and FAWPQYLK f(189–197). This bioactive peptide sequence is likely to possess antibacterial activity.

The antibacterial activity of the extraction of Sephadex G-25 from African goat milk fermented with *B. longum* on agar media containing *L. acidophilus* can be seen in **Figure 14**. **Figure 14** shows the antibacterial activity in the form of inhibiting the growth of *L. acidophilus* bacteria by extracting Sephadex G-25 from African goat milk fermented by *B. longum*. The highest inhibitory activity occurred in goat milk fermented for 12 hours, with a diameter of 1.47 mm, and lasted up to 36 hours of incubation. Bacteriostatic activity stopped after 36 hours, and bacteria in the MRSA medium began to grow. Fermentation time has no significant effect (p>0.05) on the diameter inhibition.

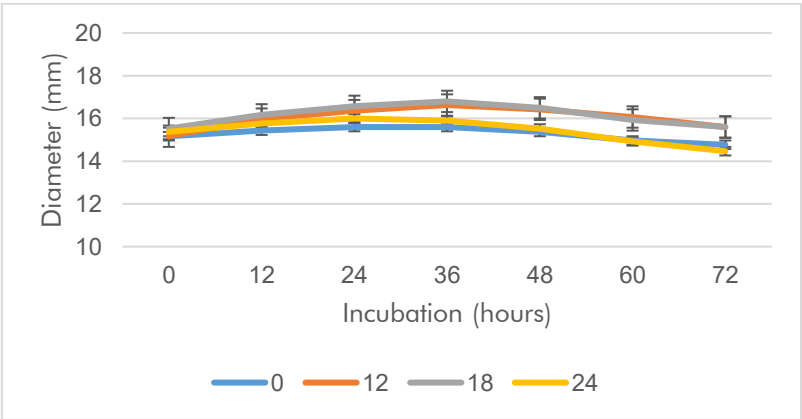

Figure 14. Inhibition zone of fermented African goat milk with *B. longum* in a medium *L. acidophilus.* Inhibition zone fermented African goat milk with *B. longum* in a medium *L. acidophilus* (P<0.05)

Based on the results of antibacterial testing of fermented goat Kacang milk and African with *L. acidophilus* and *B. longum* bacteria using the well diffusion method, with bacteria crossed between the two showed bacteriostatic antibacterial activity for up to 36 hours. According to (Georgieva et al., 2015), the zone of inhibition of Lactobacillus and Bifidobacterium spp. considered positive at a minimum diameter of 10 mm. The diameter of the inhibition zone in the research that has been carried out is the highest, in *L. acidophilus* fermented milk, which was tested on media containing *B. longum* at 1.77 mm, so it can be said to be bacteriostatic. The diameter of the wells stopped expanding and moved towards the center of the wells after 36 hours of incubation, indicating that the growth of bacteria inoculated on MRSA media was inhibited. LAB species exert a protective effect against microorganisms by producing bacteriocins, organic acids, other compounds, and/or combinations thereof (Sharma et al., 2021). *Lactobacillus* can multiply effectively in the intestinal mucosa, regulate the balance of microbiota in the intestinal epithelium, improve the gastrointestinal barrier function, inhibit the growth of potential pathogens, and prevent colon cancer (Chull-An et al., 2020). Bifidobacterium can inhibit the growth of various pathogens in the intestinal tract to reduce the formation of some pathogeneses, in addition to producing bacteriocins, acetic acid, and lactic acid to lower the environmental pH value, thereby killing pathogenic microorganisms in the human body (Yu et al., 2023).

CONCLUSIONS

The antibacterial activity of goat milk fermented with *L. acidophilus* and goat milk fermented with *B. longum* was bacteriostatic, with the highest inhibition in Kacang goat milk fermented with *L. acidophilus* for 12 until 36 hours. Lactic acid bacteria in fermented milk have a commensal relationship with bacteria in MRS media. Lactic acid bacteria in MRS media are able to withstand the antibacterial properties produced by the goat milk fermentation process.

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