Biology, Medicine, & Natural Product Chemistry ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 1129-1135 | DOI: 10.14421/biomedich.2025.142.1129-1135 ISSN 2540-9328 (online)

Beneficial Effect of Sumbawa Wild Horse Milk Yogurt on Lipid Profile and Cardiovascular Risk in Rats on a High-Cholesterol Diet

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Manuscript received: 06 October, 2025. Revision accepted: 29 November 2025, 2025. Published: 03 December, 2025.

Abstract

Cardiovascular disease (CVD) is a leading cause of death in Indonesia, with a high prevalence due to a high-cholesterol diet that triggers hypercholesterolemia. The use of statins as a long-term pharmacological therapy often causes side effects, so safe natural alternatives are needed. Sumbawa wild horse milk is a potential functional food source that contains essential fatty acids and lactic acid bacteria (LAB), which have probiotic potential. This study aims to evaluate the effect of Sumbawa wild horse milk yogurt on total cholesterol, HDL, LDL, Atherogenic Index (AI), and Cardiac Risk Ratio (CRR) levels in hypercholesterolemic male Wistar rats. A total of 30 rats were divided into six groups: normal, positive control (high-cholesterol diet), negative control (simvastatin), and three treatment groups, T1, T2, and T3, with doses of Sumbawa wild horse milk yogurt of 2, 3, and 4 ml/day, respectively, for 28 days. The results showed a significant decrease in total cholesterol levels ($p = 0.006$), with the lowest levels observed in the T3 treatment group (95.25 ± 20.17 mg/dL). Although HDL, LDL, IA, and CRR levels did not show significant differences ($p > 0.05$), a trend of improvement in lipid profiles was observed in the treatment group, characterized by an increase in HDL and a decrease in LDL, AI, and CRR compared to the positive control. The mechanism of cholesterol reduction is thought to be mediated through the activity of LAB, which assimilates cholesterol, facilitates bile acid deconjugation, and produces short-chain fatty acids that inhibit cholesterol absorption. Thus, Sumbawa wild horse milk yogurt has the potential to be a hypocholesterolemic functional food that can improve lipid profiles and reduce the risk of atherosclerosis and CVD.

Keywords: Cardiovascular disease; Cholesterol; Horse milk; Lipid profile; Yogurt.

Abbreviations: Cardiovaskular Disease (CVD), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Atherogenic Index (AI), Cardiac Risk Ratio (CRR).

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death in Indonesia, accounting for approximately one-third of total deaths. Data from the World Health Organization (WHO) over the past two decades indicates that the number of deaths due to CVD globally has reached more than 350 million cases (Velissaridou et al., 2024). In Indonesia, the number of CVD sufferers is estimated to reach around six million by 2024 and is projected to continue to increase. The 2022 National Health Survey showed that more than 25% of the Indonesian population has cardiovascular risk factors (Qanitha et al., 2022). The prevalence of CVD increases by up to 33% in individuals with high-fat diets and who are at high risk due to hypercholesterolemia (Adisasmito et al., 2020; Zakaria et al., 2022). One of the primary factors contributing to CVD is hypercholesterolemia, a lipid metabolism disorder characterized by elevated levels of total cholesterol and low-density lipoprotein (LDL), as well as reduced levels of high-density lipoprotein (HDL) (Kaneko et al., 2021). A high-cholesterol diet can lead to the accumulation of LDL in tissues, triggering oxidative stress and the development of atherosclerosis (Nugraheni et al., 2023). Cholesterol management is generally performed with pharmacological therapy, one of which is the use of statins. However, long-term use of statins carries the risk of side effects such as hepatotoxicity, myopathy, and kidney failure (Oktavelia & Kusuma, 2022). Therefore, saver and more effective alternatives for controlling cholesterol levels are needed.

Currently, functional foods have become part of the daily diet, potentially reducing the risk of various diseases. One local resource with potential as a functional food is the milk of wild Sumbawa horses. This product is part of the cultural heritage and traditions passed down through generations of the people of West Nusa Tenggara, particularly on the island of Sumbawa. It is a minor dairy product that is currently being developed in line with the increasing socio-economic role of horses in the geographic areas where they reside. It contains high nutritional value and offers health benefits. However, scientific evidence regarding its anti-inflammatory, antibacterial, and probiotic potential is still limited (Prastyowati, 2021). Mare's milk contains higher levels of essential fatty acids such as linoleic acid, particularly α-linoleic acid (ALA), capric acid, lauric acid, myristic acid, palmitic acid, oleic acid, and linolenic acid, which play a role in lowering cholesterol levels (Czyżak-Runowska et al., 2021). In addition, Sumbawa wild horse milk also contains Lactic Acid Bacteria (LAB), which have the potential to act as probiotics, with the primary function of lowering cholesterol levels and improving lipid profiles (Prastyowati, 2021). However, Sumbawa wild horse milk has low sensory quality, so research by Fajriani et al. has developed Sumbawa wild horse milk into Sumbawa wild horse milk yogurt (Fajriani et al., 2023). Fajriani et al.'s research found that Sumbawa wild horse milk yogurt is more acceptable in terms of sensory properties and contains a high total LAB count, specifically 3.42 x 10^9 CFU/mL (10). LAB is known to have the ability to assimilate cholesterol through a binding mechanism to the bacterial cell membrane, resulting in a decrease in dietary cholesterol absorption in the digestive system, which in turn decreases blood cholesterol levels. Evaluation of reduced cholesterol and LDL levels and increased HDL can be used as an indicator of CVD risk, where HDL levels >60 mg/dL indicate a lower CVD risk, as shown through the calculation of the Atherogenic Index (AI) and Cardiac Risk Ratio (CRR) (Velissaridou et al., 2024).

MATERIALS AND METHODS

Study area

This research was conducted at the Pharmaceutical Research and Development Laboratory of the University of Mataram in July–August 2025 and has received an ethical approval letter with number 058 / EC-04 / FK-06 / UNIZAR / VI / 2025. This research employed a quantitative experimental method with a completely randomized design, followed by a significant difference test for unpaired groups. The sample consisted of 30 male Wistar rats, aged 2-3 months and weighing 150-250 g. The sample was then divided into six groups: the Normal Control Group (NC), Positive Control (C+), Negative Control (C-), Treatment 1 (T1), Treatment 2 (T2), and Treatment 3 (T3), each consisting of 5 rats.

Procedures

The process of making Sumbawa Wild Horse Milk Yogurt

Yogurt production was carried out in the Food Microbiology Laboratory and the Bioprocess Laboratory of the University of Mataram. This process began with the provision of Sumbawa wild horse milk, then the addition of 5% skim milk. Next, it was homogenized at room temperature using a homogenizer for 10 minutes. After that, it was pasteurized using a water bath at a temperature of 65 °C for 20 minutes. Then, it was left to stand until it reaches a temperature of 37 °C. Inoculate a 4% starter culture containing *Lactobacillus rhamnosus* bacteria. Next, incubated at 37 °C for 24 hours. After the incubation process is complete, liquid yogurt will be obtained. The yogurt is then added with 1% honey and stored at a temperature of 10 °C (Fajriani et al., 2023).

The process of making High-Cholesterol Diet (HCD)

A high-cholesterol diet was prepared by mixing 83 g of egg yolk, 10 g of animal fat, 5 g of sucrose, 1.5% cholesterol, and 0.5% propylthiouracil until the mixture was thoroughly combined. The high-cholesterol diet was then administered at a rate of 2 ml/200 g of rat body weight (Yang et al., 2019a).

The process of making simvastatin suspension

Simvastatin was administered orally to experimental animals by preparing a suspension using carboxymethylcellulose (CMC). The preparation process began by dissolving 1 g of CMC in 100 mL of warm water, stirring until a thick, homogeneous solution was formed. Afterward, the amount of 20,6 mg simvastatin was disolveud in 100 mL of CMC suspension. The suspension was administered to the rats was carried out using the sonde method at 0,206 mg/200 g BW/day.

The treatment of experimental animals

In this study, the experimental animal subjects were first adapted for 7 days. During the adaptation process, the experimental animal subjects were given standard diet (SD). Then, they were given high-cholesterol diet (HCD) for 14 days (from day 8 to day 21), except for the normal group. After that, treatment was given for 28 days (days 22 to 49) according to the treatment group. The following is the treatment given to each group:

Table 1. The treatment of experimental animals.

Group	Days 1-7	Days 8-21	Days 22-49
NC	SD	SD	SD
C+	SD	SD and HCD	SD and HCD
C-	SD	SD and HCD	SD and 0,36 mg/200 g BW/day of simvastatin
T1	SD	SD and HCD	SD and 2ml/200 g BW/day of Sumbawa wild horse milk yogurt
T2	SD	SD and HCD	SD and 3ml/200 g BW/day of Sumbawa wild horse milk yogurt
T2	SD	SD and HCD	SD and 4ml/200 g BW/day of Sumbawa wild horse milk yogurt

The cholesterol level, HDL, LDL, IA, and CRR measurement

On the 50th day, the mice were anesthetized with ketamine, and blood was drawn through cardiac perfusion. Blood samples were placed in vacutainer tubes and centrifuged to obtain serum. Total cholesterol, HDL, and LDL levels were measured using a Biosystem machine at the Awet Muda Narmada Hospital Laboratory. This device is a semi-autoanalyzer used for clinical chemistry examinations. The IA and CRR analyses compare total cholesterol to HDL levels. The following is the formula for calculating AI and CRR:

$$IA = \frac{(Cholesterol\,Total - HDL)}{HDL}$$

$$CRR = \frac{Cholesterol\,Total}{HDL}$$

Data analysis

The results of the cholesterol, HDL, LDL, IA, and CRR level measurements were analyzed for normality using the Shapiro-Wilk test and for homogeneity using Levene's Test. HDL, LDL, AI, and CRR data were normally distributed and homogeneous, allowing for the analysis to be carried out using one-way ANOVA with a 95% confidence level (α = 0.05). Meanwhile, for total cholesterol data, it was not normally distributed and not homogeneous; therefore, the analysis was carried out using the *Kruskal-Wallis* test. If the results were significant, a further *Mann-Whitney* test was conducted.

RESULTS AND DISCUSSION

Table 2 presents the average results for total cholesterol, HDL, LDL, IA, and CRR in all groups of mice. Based on these results, there was a decrease in total cholesterol, LDL, AI, and CRR levels except in the C+ group. This is because the C+ group was only given a high-cholesterol diet. Meanwhile, HDL levels generally increased, except in the C-group. The decrease in HDL levels in the C-group was caused by the administration of simvastatin, which inhibits HMG-CoA reductase, resulting in reduced hepatic cholesterol and, consequently, decreased lipoprotein secretion, including HDL (Palmado et al., 2024).

Table 2. Average of cholesterol total, HDL, LDL, IA, and CRR.

Group	Cholesterol total (mg/dL)		HDL (mg/dL)		LDL (mg/dL)		AI (mg/dL)		CRR (mg/dL)	
	Mean±SD	P	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p
CN	105±2.00	0,006*	43.12±5.77	0,731	40.47±8.41	0,790	1.46±0.26	0,898	2.46±0.26	0,902
C+	134.50±38.45		53.87±18.68		69.62±48.28		1.78±1.40		2.78±1.40	
C-	104.25±0.50		39.97±6.36		57.15±12.67		1.66±0.44		2.66±0.44	
T1	103.75±0.95		46.22±9.54		50.02±8.59		1.35±0.50		2.60±0.47	
T2	102.25±0.95		47.42±13.96		43.41±15.05		1.33±0.70		2.33±0.70	
T3	95.25±20.17		48.00±13.74		41.03±26.55		1.19±0.85		2.19±0.85	

Note: *p-value <0,05

In general, total cholesterol levels in all groups decreased, ranging from 134.50 ± 38.45 mg/dL to 95.25 ± 20.17 mg/dL. The C+ group had the highest cholesterol levels (134.50 ± 38.45 mg/dL), while the T3 group had the lowest (95.25 ± 20.17 mg/dL). The *Kruskal-Wallis* test results in Table 2 show a p-value of 0.006, which means there are at least two groups with different total cholesterol levels. Therefore, a further *Man-Whitney* test was conducted to determine the differences in each group. The results of the further *Man-Whitney* test in Table 3 show that the CN group has a p-value <0.05 (p=0.017) compared to the T2 group, indicating significant differences in cholesterol levels between the CN and T2 groups. In addition, other groups with significant differences included C+ with C- (p = 0.018), C+ with T1 (p = 0.020), C+ with T2 (p = 0.020), and C+ with T3 (p = 0.028), as well as C- with T2 (p = 0.017). These results demonstrate that administering Sumbawa wild horse milk yogurt can reduce total cholesterol levels to near-normal levels. These results align with the research findings of (Lestari et al., 2020), which demonstrated that administering purple sweet potato yogurt for four weeks can reduce total cholesterol levels in mice. Meanwhile, consuming a high-cholesterol diet can lead to an imbalance in metabolism and fat absorption, thereby affecting the fat oxidation mechanism, which in turn can cause fat accumulation and increase cholesterol and LDL level (Abdullah et al., 2023).

Table 3. Post hoc Man-Wihtney.

Group	Group	Post Hoc
CN	C+	0.076
	C-	0.850
	T1	0.278
	T2	0.017*
	T3	0.766
C+	C-	0.018*
	T1	0.020*
	T2	0.020*
	T3	0.028*
C-	T1	0.350
	T2	0.017*
	T3	0.363
T1	T2	0.065
	T3	0.372
T2	T3	0.243

Note: *p-value <0,05

Table 2 shows that the HDL parameter showed no significant results between groups with a p-value of 0.731. However, there was a trend towards increased HDL levels in the treatment group compared to the C- group. The C+ group had the highest value at 53.87 ± 18.68 mg/dL. Meanwhile, the C- group had the lowest HDL levels at 39.97±6.63 mg/dL. This suggests that the treatment tends to increase HDL levels, and there is potential for improvement in lipid profiles as the dose increases. However, this has not yet reached statistical significance. The increase in HDL in the treatment group may indicate the role of LAB in improving lipid profiles by increasing reverse cholesterol transport from peripheral tissues to the liver for excretion (Rosenson et al., 2016). Meanwhile, the high HDL in the C+ group may be due to the body's compensatory mechanism for increased total cholesterol, which attempts to increase HDL to compensate for impaired lipid metabolism (Lodha & Kakadiya, 2022). Furthermore, the increase in HDL in the group given a high-cholesterol diet also occurred due to the redistribution of cholesterol between lipoproteins. When LDL and VLDL reach their maximum cholesterol-carrying capacity, excess cholesterol can be transferred to HDL, thereby balancing plasma lipid distribution. This is consistent with HDL's flexible role in accepting excess cholesterol from other lipoprotein (Fernandez & Murillo, 2022).

LDL levels also showed insignificant results between groups with a p-value of 0.790. The highest value was shown in the C+ group at 69.62±48.77 mg/dL. Meanwhile, the T3 group showed the lowest result at 41.03 ± 26.55 mg/dL. Although not statistically significant, these results suggest the potential for improving lipid profiles by reducing LDL levels through treatment. These results align with research by (Yang et al., 2019b), which demonstrated that administering frozen yogurt can lower LDL levels in mice.

The Atherogenic Index (AI) is a parameter used to predict the risk of atherosclerosis (Niroumand et al., 2015). Based on Table 2, the results of the AI analysis show no significant differences between groups with a p-value of 0.898. However, a downward trend was observed in the treatment group. The highest AI value was in the C+ group at 1.78±1.78, while the T3 group had the lowest value at 1.19±0.85. This decrease indicates that the treatment was effective in reducing the IA value. The lower the AI value, the lower the potential risk of atherosclerosis; however, the effect was not statistically significant (Kwarteng & Laing, 2025).

The Cardiac Risk Ratio (CRR) is a parameter that indicates the risk of CVD (Niroumand et al., 2015). The CRR parameter showed insignificant results with a p-value of 0.902. However, there was a tendency for a decrease in the CRR value in the treatment group compared to the C+ group. The C+ group had the highest CRR value of 3.78 ± 2.10, while the T3 group had the lowest value of 2.19 ± 0.85. This trend suggests that a lower CRR value may have the potential to reduce the risk of cardiovascular disease, although the difference is not statistically significant (Mansoori et al., 2024).

Discussion

Cholesterol is a macronutrient that plays a vital role in the formation of vitamin D, steroid hormones, and bile acid synthesis. Cholesterol analysis showed differences between groups. The highest cholesterol and LDL levels were observed in the C+ group, suggesting that a high-cholesterol diet can induce elevated cholesterol and LDL levels in mice. High-cholesterol diet intake, such as duck egg yolks and animal fats, contributes to increased cholesterol levels, especially the LDL fraction. These results align with research by (Shihab et al., 2023), which found that feeding a high-fat diet to mice significantly increased cholesterol, TG, and LDL levels. High-fat diets can lead to an imbalance in fat metabolism and absorption, which affects the fat oxidation mechanism and may contribute to fat accumulation (Abdullah et al., 2023). The administration of Sumbawa wild horse milk yogurt to the treatment group significantly reduced cholesterol levels in mice, with increasing doses of yogurt resulting in progressively lower cholesterol levels. The lowest cholesterol levels were found in the 4 mL of Sumbawa wild horse milk yogurt. This decrease in cholesterol levels may be due to the presence of LAB in the yogurt. The LAB in yogurt can lower cholesterol levels through several mechanisms: first, by assimilating cholesterol in the intestine through bacterial action, thereby reducing its availability for absorption. Second, the activity of bile acid deconjugation by enzymes produces an insoluble form of bile acid, which is excreted in the feces, allowing the body to utilize endogenous cholesterol for the synthesis of new bile acids. Third, the production of short-chain fatty acids can inhibit cholesterol absorption in the intestine. Furthermore, yogurt also contains bioactive peptides that play a role in reducing oxidative stress, suppressing LDL oxidation, and improving lipid metabolism (Kumar et al., 2012).

Administering Sumbawa wild horse milk yogurt to mice has been shown to lower LDL levels. This mechanism aligns with several studies showing that fermented milk products can have a hypolipidemic effect. The fermentation process produces bioactive compounds, enzymes, and microorganisms that have the potential to act as probiotics and contribute to lowering LDL levels in the blood (Prastyowati, 2021; Ziaei et al., 2021). Furthermore, fermented Sumbawa wild horse milk can also increase the production of Short Chain Fatty Acids (SCFA), particularly propionate, which is known to inhibit the activity of HMG-CoA reductase in the liver, a key enzyme in cholesterol biosynthesis. Horse milk also has a lower fat content and a different fatty acid profile than cow's milk, thus naturally supporting a better blood lipid profile. The combination of fermented LAB, bioactive metabolites, and the characteristics of horse milk contributes to lowering LDL. Conversely, in groups with a high-cholesterol diet, excessive intake of saturated fat and cholesterol leads to increased LDL levels due to disruption of cholesterol homeostasis, including an increase in the synthesis of atherogenic lipoproteins (Ziaei et al., 2021). Increased cholesterol content in food and its absorption in the intestine can stimulate the liver to produce large amounts of LDL molecules, which transport triglycerides and cholesterol to body tissues through the bloodstream (Alcover et al., 2025).

The increase in cholesterol and LDL levels in the C+ group was directly correlated with the increase in HDL levels in the same group. The high HDL levels in the group given a high-cholesterol diet may be due to the redistribution of cholesterol between lipoproteins. When LDL and VLDL reach their maximum cholesterol-carrying capacity, excess cholesterol can be transferred to HDL, thereby balancing plasma lipid distribution. This is in line with HDL's flexible role in accepting excess cholesterol from other lipoproteins (Fernandez & Murillo, 2022). Furthermore, it is suspected that this is due to the body's compensatory mechanism for increased total cholesterol, which causes the body to attempt to increase HDL to offset impaired lipid metabolism (Lodha & Kakadiya, 2022). The lowest HDL levels were in the C-group. Simvastatin administration to the C- group inhibited HMG-CoA activity, thus inhibiting cholesterol synthesis. Statins are drugs that lower cholesterol by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which plays a role in cholesterol production in the liver (Tucker & Soslowsky, 2016).

Elevated cholesterol and LDL levels will affect the atherogenic index (AI) value. An elevated Atherogenic Index (AI) reflects the occurrence of atherogenic dyslipidemia, characterized by high cholesterol levels, low HDL, and the formation of small, dense LDL particles. Small-dense LDL is more atherogenic than large LDL because it easily penetrates the vascular endothelial layer, is more susceptible to oxidation, and remains in circulation longer (Gaggini et al., 2022). Atherosclerosis itself is the leading cause of CVD. It is considered a chronic inflammatory disease resulting from the interaction of LDL and the arterial wall, leading to the formation of atherosclerotic lesions. Furthermore, a high-cholesterol diet also triggers oxidative stress and a systemic inflammatory response, which exacerbates endothelial dysfunction and further increases AI values (Vinué et al., 2018). The relationship between AI and CVD has been demonstrated in various clinical and epidemiological studies. The results of a meta-analysis showed that high AI is an independent predictor of coronary heart disease events and can serve as a more sensitive prognostic indicator than individual lipid parameters (Rad et al., 2024). High AI indicates the dominance of pro-atherogenic factors in lipid metabolism, which ultimately increases the risk of atherosclerosis, plaque susceptibility, and clinical events such as myocardial infarction or stroke (Młynarska et al., 2024). Groups of mice fed different doses of Sumbawa wild horse milk yogurt showed a reduced atherogenic index compared to the high-cholesterol diet group. This mechanism is closely related to the bioactive composition of wild horse milk and the potential activity of fermented probiotics. Horse milk yogurt contains whey protein, bioactive peptides, unsaturated fatty acids, and microorganisms with potential probiotic properties known to play a role in modulating lipid metabolism (Prastyowati, 2021). Unsaturated fatty acids, such as linoleic and linolenic acids, can increase lipoprotein lipase activity, thereby facilitating the breakdown of triglycerides. These fatty acids also play a role in increasing HDL concentrations, which can directly reduce the atherogenic index (Figueiredo et al., 2017). Horse milk yogurt also has potential antioxidant and anti-inflammatory effects, as the fermentation process produces bioactive compounds, including antioxidant peptides and organic acids, that can reduce oxidative stress and suppress vascular inflammation. This is important because oxidative stress and chronic inflammation contribute to changes in HDL quality, leading to dysfunction. By maintaining HDL's protective function, yogurt helps maintain low AI levels (Pânzaru et al., 2024; Titisari et al., 2020).

High cholesterol levels are also a risk factor for cardiovascular disease (CVD). Apolipoprotein B100 (ApoB100) is the main structural protein of atherogenic lipoproteins, whose increase is associated with increased LDL, thus increasing the risk of CVD, as indicated by an increased CRR value (Pourrajab et al., 2020). The study results showed that the highest CRR value was in the C+ group. This may be due to the high-cholesterol diet increasing cholesterol and LDL levels. The higher the CRR value, the higher the risk of CVD. Conversely, the lower the CRR value, the lower the risk of CVD.

CONCLUSIONS

This study showed that Sumbawa wild horse milk yogurt has the potential as a hypocholesterolemic functional food that can improve blood lipid profiles and reduce the risk of CVD. Administering yogurt at doses of 2 ml, 3 ml, and 4 ml per day for 28 days in hypercholesterolemic male Wistar rats significantly reduced total cholesterol levels ($p = 0.006$), with the lowest result at a dose of 4 ml/day (T3). Although HDL, LDL, Atherogenic Index (AI), and Cardiac Risk Ratio (CRR) levels did not show significant differences between groups ($p > 0.05$), there was a trend of increasing HDL and decreasing LDL, AI, and CRR in the treatment group compared to C+. The mechanism of cholesterol reduction is thought to originate from the activity of LAB, which assimilates cholesterol, bile deconjugation, and produces short-chain fatty acids that inhibit cholesterol absorption. In addition, the content of unsaturated fatty acids and bioactive peptides in Sumbawa wild horse milk also plays a role in suppressing LDL oxidation, increasing lipoprotein lipase activity, and providing antioxidant and anti-inflammatory effects. Thus, Sumbawa wild horse milk yogurt can be a safe and effective natural alternative for lowering cholesterol levels and reducing the risk of atherosclerosis, without causing side effects associated with long-term statin therapy.

Acknowledgements: The author would like to thank the Ministry of Higher Education, Science, and Technology (Kemdiktisaintek), Bumigora University, and all colleagues who helped in carrying out this research.

Authors' Contributions: Laksmi Nur Fajriani designed the study. Laksmi Nur Fajriani and Ni Made Wiasty Sukanty carried out the laboratory work. Laksmi Nur Fajriani analyzed the data and wrote the manuscript. Dea Pratiwi Silpia Putri and Esya Mariska Febriani were involved in collecting Sumbawa wild horse milk for yogurt production and assisting in the production of Sumbawa wild horse milk yogurt. All authors read and approved the final version of the manuscript

Competing Interests: The authors declare that there are no competing interests.

Funding: This research was funded by the Ministry of Higher Education, Science, and Technology (Kemdiktisaintek) through the Research and Community Service (BIMA) Program in 2025.

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