Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 5, 589-594

Goat milk whey fermentation: A sustainable approach to producing tryptophan functional beverages by *Lactobacillus rhamnosus* and *Lactobacillus casei*

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ARTICLE INFO

Recieved: 21 August 2025

Accepted: 17 October 2025

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Keywords:

Fermented whey, Food sustainability, *Lactobacillus casei* , *Lactobacillusrhamnosus* , Tryptophan

ABSTRACT

The challenge of sustainable goat milk processing can be partially overcome by utilizing the nutrients in whey to create functional foods that can improve health. This encourages the utilization of nutrients in goat milk whey through fermentation by utilizing microbes to produce bioactive compounds, one of them is tryptophan. Protein in whey one of which is lactalbumin, rich in the essential amino acid tryptophan. This study examines the fermentation of goat milk whey using *L. rhamnosus* and *L. casei*, to assess tryptophan levels, along with chemical and microbiological properties Sweet whey was produced by using rennet, centrifugated and filtrated, then inoculated with these bacteria according to the treatment (*L. rhamnosus* : *L. casei* = 1:0; 0:1; 1:1). Incubation occurred at 37°C for a duration of 24 hours. The results showed that starter culture affects significantly ($P<0.05$) to lactose, pH, crude protein and crude tryptophan; but did not affects significantly ($P>0.05$) to titratable acidity and lactic acid bacteria count. In conclusion, fermented whey contained nearly lactose 3.39%-4.41%; pH 3.94-4.00; acidity 0.06%-0,11%; lactic acid bacteria count 9.55-9.89 log CFU/ml; crude protein 12.24%-19.57% and crude tryptophan 89.72 - 113.98 mg/100mg.

Introduction

Indonesia ranks among the top 10 nations globally for having the largest stock of dairy goats, namely 3.74% and contributes to the global goat milk product with 2.09% (Meza-Herrera *et al*., 2024). Currently, in Indonesia, the trend of consuming goat's milk is increasingly popular because it has advantages over cow's milk in several ways. It has high digestibility and buffer capacity as well as different alkalinity (Park *et al*., 2017). According to Cahyanti and Legowo (2024), this milk has low lactose, protein and fat characteristics, certain mineral and vitamin levels that are different from cow's milk. So it does not cause allergic reactions, has an impact on the functional value, therapeutic effects and bioavailability of certain minerals and vitamins. It is useful as a dietary supplement for those who have special nutritional needs.

According to Sumarmono (2022), eco-freindly dairy goat farming has been established in Indonesia, which is its production being 0.28 gallon/d or 50,66 gallon per lactation on average, utilized for drinking directly and a range of fermented dairy items like cheese . Although the industrial manufacturing of goat-milk based cheese in Indonesia remains uncharted, a feasibility study has been conducted regarding the cheddar cheese industry based on goat milk in Indonesia (Nursanto *et al*., 2022). Reportedly, some goat milk processing into goat cheese is in Boyolali, Sleman, Lumajang, and Bali. From the cheese making, goat milk whey is produced which has not been specifically recorded and requires further processing because it has the potential to pollute the environment with BOD values (> 80g/L) and COD (> 60g/L) that do not meet the permitted safe limits (Febrisiantosa *et al*., 2013). Thus, the challenge of sustainable goat milk processing can be partially overcome by utilizing the nutrients in whey to create functional foods that can improve health. This encourages the utilization of nutrients in goat milk whey through fermentation.

As stated by Abd-Elatif *et al*. (2023), at the present time, nutritious functional foods that include bacteria with beneficial health effects are-gaining popularity and fermented dairy items are increasingly taking a prominent position in consumers' preferences Supported by several previous studies, whey as one of the dairy products has been successfully utilized as a functional drink by utilizing microbes to produce bioactive compounds (Saglam *et al*., 2019; Melia *et al*., 2022; Kaur *et al*., 2022; Dinkçi *et al*., 2023). These compounds have naturally been enhanced through special bacterial growing conditions as reported by Kumar *et al*. (2012). Exploration of goat whey has been carried out in order to develop functional foods to help various degenerative diseases and psychiatric diseases (Ahmed *et al*., 2020; Dinkçi *et al*., 2023; Moslemi *et al*., 2023, Rosa *et al*., 2023; Tlais *et al*., 2023).

Whey consists of two main proteins, namely β-Lactoglobulin at 50-55% and α-Lactalbumin at 20-25%. These proteins contribute to the functional properties of whey protein (Elaziz *et al*., 2024). Lactalbumin is a protein that has a molecular weight (MW) of 14.2 kDa that binds to Ca2+ (Copriyadi *et al*., 2011). Lactalbumin is rich in the essential amino acid tryptophan (Kamau *et al*., 2010; Layman *et al*., 2018). However, several studies have investigated tryptophan and its derivative metabolites present in milk and dairy products (Bertazzo *et al*., 2016; Yilmaz and Gokmen, 2018; Su *et al*., 2020).

In humans and animals, tryptophan is not synthesizable because they do not have tryptophan synthase, thus it must be acquired through food. It will be crucial not only for body growth but also to carry out various metabolic functions of the body (Ritota and Manzi, 2020).

Tryptophan and its metabolites play significant biological processes in humans , needed for vital physiological and neurological function. It is involved in protein synthesis and serves as a precursor for numerous biologically active compounds in the body. . It can be metabolized into bioactive substances that have benefits to improve human health. Arvidsson *et al*. (2007), stated that tryptophan plays a role in the development of various neurological disorders such as schizophrenia, Alzheimer and Parkinson diseases, epilepsy as well as multiple sclerosis. . Tryptophan is a precursor of serotonin which is responsible for the occurrence of anxiety and depression (Gladson *et al*., 2022). Tryptophan is also a precursor for

ISSN: 2090-6277/2090-6269/

niacin, has anti-inflammatory and antioxidant properties, which may be beneficial in treating obesity caused by emotional eating disorder and atherosclerosis (Zhu *et al*., 2024). The metabolism of tryptophan follows serotonin pathway that produces serotonin, melatonin, and 5-hydroxy-tryptophan that can improve sleep, reduce irritability, and bring feelings of happiness; as well as indole pathway which forms indole formaldehyde, indole acetic acid, indole-3-acetamide, and tryptamine, among others; some of these metabolites have immune regulation functions (Su *et al*., 2020). Moreover, Nayak *et al*. (2019), stated that the development of behaviour and cognition in children is affected by tryptophan availability.

Tryptophan can be produced by microbes. Several studies have stated that several bacteria included in *Lactococcus* sp, *Lactobacillus* sp, *Streptococcus* sp, *Escherichia coli* and *Klebsiella* sp have been reported to be able to express tryptophan synthetase and produce serotonin. Probiotic bacteria and lactic acid bacteria have the potential as probiotics and are known to play a role in the synthesis of tryptophan in the intestine and its metabolism. Jeong *et al*. (2021), stated that several strains of Lactiplantibacillus plantarum can be used as functional probiotics for stress regulation. The strain has a biosynthetic pathway to produce tryptophan, acting as a precursor for serotonin. Meanwhile, several researchers have stated that *Lactobacillus rhamnosus* (Kamil *et al*., 2021; Vinderola and Reinheimer, 2000;Yao *et al*., 2019) and *Lactobacillus casei* (Olimpio *et al*., 2023;) have proteolytic properties that can produce various free amino acids as a result of their metabolism.

Tryptophan research could contribute to developing better foods or beverages to meet these specific nutritional needs. Considering that whey is one of the most important sources for tryptophan intake, it is necessary to investigate its contents in fermented whey and its chemical characteristics at once to provide references for developing fermented whey as a functional beverage.

Materials and methods

Raw goat milk were collected from "Nio Farm" Organic Farm, Semarang, Indonesia. Commercial rennet and citric acid purchased from Cheese Making Supply, Surabaya, were in powder form and food grade. The chemicals used in this study were of analytical grade and sourced from Merck and Oxoid Companies.

Microorganisms used and culture conditions

The two isolates were *Lactobacillus* rhamnossus Collins *et al*. (*Lactobacillus casei* subsp. *rhamnosus*) FNCC 0052 obtained from Food and Nutrition Study Center (FNSC) Universitas Gadjah Mada, Yogyakarta and *Lactobacillus casei* Shirota strain from commercial product Yakult from Yakult Honsha.Co.Ltd. The culture of these bacteria was initiated by streaking onto their specific media - deMan Rogosa and Sharp (MRS) (OXOID CMO361 brand), then incubated at 37°C for 24 hours.

Starter preparation and whey fermentation

The all strains were initially grown to achieve 10^7, respectively, with subsequent subcultures in MRS Broth at 37°C for 24 hours then in skim milk media and incubated at 37°C for 48 hours for *L. casei* and 24 hours for *L. rhamnosus*. Sweet whey is made from fresh goat milk, pasteurized at 75°C for 15 seconds, then cooled to 35°C, and added 0.2% (w/v) citric acid. Subsequently added with 0.0012% (w/v) rennet in a closed container and incubated at 30°C for 60 minutes to form curd (Setiadji *et al*., 2018). The curd was filtered using a stainless steel strainer and cheese cloth to isolate the curd from the whey, and continued filtering using vaccum filter. The prepared whey was inoculated with 1% (10 ml/L) of mixed culture with a predetermined ratio (*L. rhamnosus*: *L. casei* = 1:0; 1:1; 2:1) and incubated at 30°C for 24 hours.

Chemical analysis

Lactose was measured using the technique of Telles (Melia *et al*., 2022), the pH value was assessed using the method established by the Association of Official Analytical Chemists. (Melia *et al*., 2022), Titratable Acidity was represented as grams of lactic acid per 100 grams (%w/w) , was determined according to Setiawati *et al*. (2019). Crude protein content was determined using method of Kjeldahl (Chang and Yang, 2017).

Microbiological examination

Enumeration of total lactic acid bacteria was performed using the pour plate technique using deMan Rogosa and Sharp (MRS) agar medium. One mililiter of whey samples were homogenized with 9 ml 0.85% physiological sodium chloride using vortex. Ten-fold serial dilutions were produced up to 10^9, plated on agar and aerobically incubated at 37°C for 24 hours as reported by Setiawati *et al*. (2019). Cell counts were expressed as log $CFUml^{-1}$.

Determination of crude tryptophan

The analysis carried out based on spectrophotometric method related with problematic due to tryptophan lability to acid hydrolysis, often a prerequisite for HPLC analysis. It was analysed as crude tryptophan because tryptophan is a fluorescing chromophore, there is another fluorescing chromophore, tyrosine, in protein hydrolysates. Concentration of crude tryptophan was determined using the method of ultraviolet absorption (Chang and Yang, 2017). Proteins exhibit significant absorption in the ultraviolet (UV) spectrum at UV 280 nm, mainly attributed to the presence of tryptophan and tyrosine residues within the proteins.

Statistical analysis

The data were alnalyzed using a One-Way ANOVA to determine the effect of differences in starter culture on tryptophan levels, chemical properties and microbiological properties of fermented goat milk whey. The mean and standard deviation "SD" of all the parameters were displayed. If the action has a notable effect, it proceeds with the Duncan Multiple Range Test (DMRT) at a significance level of 5%, evaluated using SPSS (IBM SPSS, 2017; Version 25) software in accordance with Duncan's multiple range test.

Results

Table 1 presented the analysis for lactose, titratable acidity, and pH value. The greatest amount of lactose was found in whey fermented with both *L. rhamnosus* and *L. casei*. The least amount detected in whey fermented with *L. casei*. The variance analysis revealed that the starter culture significantly impacts the lactose content ($P<0.05$). The values for *L. rhamnosus*, *L. casei*, *L. rhamnosus* : *L. casei* (1:1) were 4.12±0.04, 3.39±0.06, and 4.41±0.58, respectively. Titratable acidity reflects the quantity of lactic acid present in whey that has been fermented with bacteria according to the treatments. The effect of mixed culture on titratable acidity of fermented whey is also presented in Table 1. The highest titratable acidity was obtained in whey fermented using L casei while the lowest found in whey fermented using *L. rhamnosus*. The analysis of variance indicated that starter culture did not have a significant effect on titratable acidity ($P>0.05$). The values for *L. rhamnosus*, *L. casei*, and *L. rhamnosus* : *L. casei* (1:1) were 0.06±0.03, 0.11±0.21, and 0.08±0.05, respectively. The pH level reflects the acidity of whey fermented with bacteria based on the treatment applied. Table 1 shows that the highest pH value was recorded in whey fermented with *L. rhamnosus*, whereas the lowest was observed in whey fermented with *L. casei*. The variance analysis indicated that the starter culture significantly impacts the pH ($P<0.05$). The values

for *L. rhamnosus*, *L. casei*, and *L. rhamnosus* : *L. casei* (1:1) were 4.49±0.30, 3.94±0.21, and 4.00±0.05, respectively.

Table 1. Lactose, Titratable Acidity and pH content of whey fermented with different cultures.

Traits[1]	*L. rhamnosus*		*L. casei*		*L. rhamnosus : L. casei* (1:1)	
	value	Pr>F	value	Pr>F	value	Pr>F
L (g/100 ml)	4.12±0.04	0.04	3.39±0.06	0.05	4.41±0.58	0.04
TA (%)	0.06±0.03	0.20	0.11±0.21	0.48	0.08±0.05	0.25
pH	4.49±0.30	0.03	3.94±0.21	0.64	4.00±0.05	0.70

[1]L: Lactose; TA: Titratable Acidity

Lactic acid bacteria count indicates its population in whey after fermentation according to the treatment. The total lactic acid obtained from this study can be seen in Table 2. The variance analysis indicated that the starter culture did not have a significant impact on the count of lactic acid bacteria (P>0.05). The values for *L. rhamnosus*, *L. casei*, *L. rhamnosus*: *L. casei* (1:1) were 9.55±0.57, 9.69±0.54 and 9.89±0.58, respectively.

Table 3 demonstrates the variations in Crude protein and essential amino acid Tryptophan. Crude protein contained in fermented whey was analyzed using Kjeldahl Methode. It showed the highest protein value was obtained in whey fermented using both *L. rhamnosus* and *L. casei*, while the lowest found in whey fermented using *L. casei*. The variance analysis indicated that the starter culture significantly influences the protein (P<0.05). The values for *L. rhamnosus*, *L. casei*, *L. rhamnosus*: *L. casei* (1:1) were 15.17±0.03, 12.24±0.04, and 19.57±0.12 respectively. Crude tryptophan is available in fermented whey through bacteria capability to hydrolize protein into amino acids as well as to synthesize it. It showed the highest tryptophan value was obtained in whey fermented using both *L. rhamnosus* and *L. casei*, while the lowest found in whey fermented using *L. casei*. The variance analysis indicated that the starter culture has a significant impact on tryptophan (P<0.05). The values for *L. rhamnosus*, *L. casei*, *L. rhamnosus*: *L. casei* (1:1) were 90.85±0.99, 89.72±0.93 and 113.98±3.19 respectively.

Discussion

This study focused on selecting a starter culturecapable of producing crude tryptophan from fermentation of cheese whey from goat milk. The whey can be used as natural source of tyrptophan as bioactive compound resulting from the hydrolysis of α-lactalbumin, one of the most abundant protein fractions of whey (Kamau *et al*., 2010 and Layman *et al*., 2018). *L. rhamnosus* and *L. casei* are choosen as appropriate candidates based of the following reasons : stated as Generally Recognized as Safe/GRAS (Cheong *et al*., 2022; Aini *et al*., 2024), the ability to grow in whey (Hamme *et al*., 2009; Panesar *et al*., 2010), to use lactose as carbon source (Calasso and Gobbetti, 2011; Bidart *et al*., 2018) and have proteolytic activity (Kuo *et al*., 2024; Putri *et al*., 2023).

Before fermentation process whey has a pH value of 6.09. This is in accordance with Pires *et al.* (2021) that pH value of sweet whey obtained from coagulation using rennet, ranges from 6-7. The *L. rhamnosus* and *L. casei* starter were successfully created and the resulting populations were 8,68 log CFU/ml and 8,79 log CFU/ml. This is in accordance with Stanbury*et al*. (1995) that population requirements to be able to initiate fermentation are at least log 7 CFU/ml. Both starters are homolactic bacteria and able to grow in milk-based foods because they have the lactase enzyme which can break down lactose into lactic acid.

The highest lactose was obtained in whey fermented using both *L. rhamnosus* and *L. casei*. The lowest found in whey fermented using *L. casei*. The variance analysis indicated that the starter culture significantly impacts the lactose content (P<0.05). A prior study found that the lactose concentration in sweet whey is around 4.95%. (Ahmed *et al*., 2015), and 5,43% (Nursiwi *et al*., 2015). Lower lactose concentrations in fermented whey may indicate lactose degradation caused by among other things, bacterial metabolism. Within the bacterial cell, β-galactosidase breaks down lactose into glucose and galactose, which is then transformed into L(+)-lactic acid following the Embden-Meyerhof pathway (Valík *et al*., 2008), or through citrate metabolism to produce another organic acid such as succinate, acetate, and formate via pyruvate (Nuryana *et al*., 2019).

Lactose found to be different in fermented whey with different starter culture. It indicates there were distinctive lactase activities for each starter culture when fermentation occured at a temperature of37°C. *L. casei* is thought to have better lactase activity than *L. rhamnosus*. According to Hussain *et al.* (2021), one strain of *L. rhamnosus* does not thrive in milk because it lacks the ability to hydrolyze lactose. Valík *et al*. (2008) further clarify that *L. rhamnosus* is a facultatively heterofermentative bacterium with reduced β-galactosidase activity. This causes these bacteria has low ability to utilize lactose. Conversely, *L. casei* capable to use lactose better than *L. rhamnosus*. Data shows lower lactose content (3.39±0,04 g/100ml) in fermented whey when these bacteria are used as a starter. Douillard *et al.* (2013), stated that *L. rhamnosus* has the inability to use D-lactose whereas *L. casei* able to convert lactose.

Titratable acidity indicates the amount of lactic acid contained in whey fermented using bacteria according to the treatment. The highest titratable acidity was obtained in whey fermented using L casei while the lowest found in whey fermented using *L. rhamnosus*. The variance analysis indicated that starter culture had no significant effect on titratable acidity (P>0.05).

The total lactic acid is slightly produced in this fermentation, the amount is not as usual in fermented milk. It is suspected that lactic acid is not only the organic acid produced. Temporarily it can be concluded that decreased lactose levels is not only entirely accompanied by the formation of lactic acid. Another possible explanation is according to Liu *et al.* (2015), that an environment rich in protein and few amount of lactose like whey impacts the capacity of lactic acid bacteria to hydrolyze milk protein in the fermentation process, especially in low-carbohydrate milk products. So that in unusual conditions or under nutritional or environ-

Tabel 2. Lactic acid bacteria count of whey fermented with different cultures.

Traits[1]	*L. rhamnosus*		*L. casei*		*L. rhamnosus : L. casei* (1:1)	
	value	Pr>F	value	Pr>F	value	Pr>F
LAB (log CFU/ml)	9.55±0.57	0.51	9.69±0.54	0.19	9.89±0.58	0.31

[1]LAB: Lactic Acid Bacteria Count

Tabel 3. Tryptophan, crude protein content of whey fermented with different cultures.

Traits[1]	*L. rhamnosus*		Protein. Casei (%)		*L. rhamnosus* : *L. casei* (1:1)	
	value	Pr>F	value	Pr>F	value	Pr>F
TRP	90.85±0.99	0.05	89.72±0.93	0.03	113.98±3.19	0.05
CP	15.17±0.03	0.03	12.24±0.04	0.04	19.57±0.12	0.04

[1]TRP: Tryptophan; CP: Crude Protein

mental pressure, lactic acid bacteria will have an adaptation mechanism by breaking down protein first compared to lactose (; Leroy and De Vuyst, 2004). This is also consistent with Malos *et al*. (2025), who state that Lacticaseibacillus *rhamnosus* is recognized for its significant proteolytic activity and capacity to produce peptides. Afterwards lactic acid produced is slightly amount.

Crude protein was analyzed using Kjeldahl Methode, and it showed the highest value was obtained in whey fermented using both *L. rhamnosus* and *L. casei*, while the lowest found in whey fermented using *L. casei*. The variance analysis indicated that the starter culture has a significant impact on crude protein (P<0.05). Crude protein obtained ranged from 12.24% to 19.57%. This is considered high value according to Cho *et al*. (2015), compared to the results of their research which obtained 9% protein in whey fermented using *Lactobacillus plantarum* DK211. It is also said that the amount of protein is twice as high as commercial yogurt products and almost the same as the content of commercial protein drinks. This finding aligns with León-López *et al*. (2020), indicating that 9.3% protein was generated in whey beverages fermented with a combination of lactic acid bacteria. *L. rhamnosus, L. bulgaricus, L. delbrueckii,* and *Streptococcus thermophilus*, and Dinkçi *et al*. (2023) that protein derived from fermented whey of goat milk utilizing a mixed culture of different lactic acid bacteria (*Streptococcus thermophilus*, *L. acidophilus*, and *Bifidobacterium bifidum*) was roughly 8%.

The distinction occured is caused by different proteolytic activities by these bacteria (Putri *et al*., 2023; Kuo *et al*., 2024). *L. rhamnosus* is thought to have better proteolytic activity than *L. casei*. According to Fialho*et al*., (2023) and), *L. casei* has a proteolytic activity of 15-20 U/ml. While *L. rhamnosus* has a proteolytic activity of 10-63.3 U/ml (Kamil *et al*., 2021; Vinderola and Reinheimer, 2000; Yao *et al*., 2019). The combination of the two should produces greater proteolysis. The interaction of the two cultures produces proteins in greater quantities.

Crude tryptophan is available in fermented whey through bacteria capability to hydrolize protein into amino acids as well as to synthesize it. The amount of tryptophan can be seen obtained ranged from 89.72 mg/100g to 113.98 mg/100g. It showed the highest tryptophan value was obtained in whey fermented using both *L. rhamnosus* and *L. casei*, while the lowest found in whey fermented using *L. casei*. The analysis of variance showed that starter culture affects the crude tryptophan significantly (P<0,05). This distinction showed that the proteolytic activity of each culture is different when in whey substrate from goat milk with a fermentation temperature of 37°C. *L. rhamnosus* is thought to have better proteolytic activity than *L. casei*. Pearson correlation analysis was carried out to further determine the relationship between crude protein and crude tryptophan in whey fermentation at a temperature of 30°C using these bacteria. A notable positive correlation ($r > 0$, $p < 0.05$) was observed in the association between crude protein and crude tryptophan, with $r = 0.911$ and $p = 0.00000235$. This also shows that the higher the crude protein content, the higher the tryptophan content obtained. Crude protein consists of proteins, peptides, and amino acids, one of which is tryptophan.

The availability of tryptophan in fermented whey is the result of both its synthesize and catabolism. *L. rhamnosus* itself has a metabolic pathway to synthesize tryptophan Zeng *et al*., 2022), wherease can metabolizes through various pathways (Suntornsaratoon *et al*., 2024). *L. casei* can catabolize tryptophan thorugh a process that includes decarboxylation, transamination and dehydrogenation, ultimately producing indole-3-lactic acid (Gummalla and Broadbent, 1999).

The pH value indicates the acidity level of whey fermented using bacteria according to the treatment. Data of pH shows the highest pH value was obtained in whey fermented using *L. rhamnosus* while the lowest found in whey fermented using *L. casei*. The analysis of variance showed that starter culture affects the pH significantly (P<0,05). The distinctive pH arises from the differing metabolic capabilities of these two bacterial types at at 37°C, in their processes of converting lactose into lactic acid and other organic acids, along with degrading proteins into peptides and amino acids. In mixed culture, the final whey pH is the result of the interaction of *L. rhamnosus* and *L. casei* during growth together in utilizing the nutrients in whey as well as the response of them to fermentation environmental conditions such as temperature.

Compared to another fermented whey from goat's milk which generally have a pH value of 3 to 4, the pH value of whey fermented at a temperature of 30°C is higher or not too acidic. The main cause is the difference in optimal temperature for each starter to break down lactose. According to Akhtar *et al*. (2020), *L. casei* optimally produce lactase in 35°C, while*L. rhamnosus* at a temperature of 37°C optimal for lactic acid production when using whey permeate as a substrate (Cui *et al*., 2012). In this study, *L. casei* is able to degrade lactose better so that more acid and a lower pH are produced. This is supported by Pham *et al*. (2000) that the highest lactic acid began to be obtained at the 30th hour and was constant until the 70th hour when these bacteria were grown at a temperature of 37°C. At that time no lactose was found, indicating that the lactose had broken down into lactic acid.

Homolactic and heterolactic bacteria produce lactic acid and various types of organic acids like acetic acid, butyric acid, etc., with different amounts and compositions. Variations in the composition of acids generated in whey fermented with both bacterial types definitely influence the pH level of the fermented whey due to their interaction. One of the interactions will influence the growth rate of both bacteria, and the outcome is the resulting pH.

Lactic acid bacteria count indicates its population in whey after fermentation according to the treatment. The strains employed in this study thrived in whey and demonstrated varying levels of acidification, as indicated by the pH value. The values for *L. rhamnosus*, *L. casei*, *L. rhamnosus* : *L. casei* (1:1) were 9.55±0.57, 9.69±0.54 and 9.89±0.58 log CFU/ml respectively. The bacteria counts found in this study align with those reported by Cho *et al*. (2015), that fermented whey using *Lactobacillus plantarum* has a fairly high population of around 10^9 CFU/ml or 9 log CFU/ml. This amount is however better than another study using whey in various form that had populations remained under $\log^9$ CFU/ml, among others reconstituted whey fermented with *L. delbrueckii* subsp. *bulgaricus*, S. thermophilus and *L. acidophilus* either individually or combined in pairs (Pescuma *et al*., 2008) and reconstituted goat whey powder fermented with *S. thermophilus* paired up with *L. rhamnosus* or *L. casei* or *L. paracasei* each other (Santos *et al*., 2019). Nevertheless The variance analysis indicated that the starter did not significantly impact the count of lactic acid bacteria (P>0.05). It was assumed that whey protein did not consistently lead to superior growth in specific bacterial strains or combinations of strains. It was likely due to the same capability to utilize specific nutrients, including the amino acids in whey protein by these different bacterial strains.

Conclusion

Variations in starter culture notably influenced lactose, pH level, crude protein, and tryptophan, but did not have a significant impact on titratable acidity and total lactic acid bacteria in whey fermented with *L. casei*, *L. rhamnosus*, and a mix of *L. rhamnosus* and *L. casei*.

Acknowledgments

The authors thank the Institute of Research and Community Service, Universitas Semarang for funding this research through "Penelitian Hibah Kompetisi Internal" (Research Implementation Agreement Number: 016/USM.H7.LPPM/L/2024).

Conflict of interest

The authors have no conflict of interest to declare.

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