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Effect of the season and climatic factors on the microbiological quality of raw goat milk in Southern Thailand

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ABSTRACT

Microbial contamination of raw milk considerably affects the quality and safety of dairy products. Dairy farm environmental factors (e.g., temperature, humidity and milking hygiene) involved with the microbiological quality of raw goat milk were previously elucidated. Until now, the research on the quality of raw goat milk in Thailand is still limited. The current study focused on the variation in microbiological quality (i.e., total bacteria, coliforms, proteolytic bacteria, lipolytic bacteria and thermoduric bacteria) of raw goat milk affected by climatic factors (i.e. rainfall, relative humidity and ambient temperature). Study area included raw goat milk from smallholders in the lower part of Southern Thailand. The results indicated that contamination of total bacteria, lipolytic bacteria, proteolytic bacteria and coliforms was higher during the rainy season than in the dry season ($P<0.05$), whereas there was no statistical difference ($P<0.05$) in the thermoduric bacteria in raw goat milk collected during the dry and rainy seasons. The current study revealed a positive correlation between two climatic factors (i.e. rainfall and relative humidity) and four microbiological parameters (i.e. total bacteria, coliforms, proteolytic bacteria and lipolytic bacteria). In addition, the ambient temperature was negatively correlated with the abovementioned four microbiological parameters. No statistical association between thermoduric bacteria and three climatic factors (i.e. rainfall, relative humidity and ambient temperature) was found. To ensure consistently quality of goat milk, farmers should enhance milking hygiene practices during the rainy season, when rainfall and humidity are higher in the dry season.

Introduction

Goat milk is a dairy product defined as a natural functional food (Verruck *et al*., 2019). It is particularly favoured in low-income countries or places where cow's milk is not easily brought (Miller and Lu, 2019; ALKaisy *et al*., 2023). Compared to cow's milk, the type and proportion of certain chemical components in goat milk, such as milk fat, protein, ash and lactose, are different (ALKaisy *et al*., 2023). Goat milk utilisation is commonly related to cow's milk replacement, as a healthy food for delicate individuals and a crucial instrument to complete the 2030 Agenda for Sustainable Development Goals (SDGs) (Idamokoro, 2023). Unsurprisingly, the dairy goat industry has grown globally in the last decade (Miller and Lu, 2019). However, dairy goat production is usually operated on a small scale and associated with local consumption (Miller and Lu, 2019). Interestingly, some of the goat production sectors in Southeast Asia, including Thailand, are integrated with plantation agriculture, such as palm oil, rubber tree and fruits (Devendra, 2010; Pralomkarn *et al*., 2011). The quality of raw milk production on these types of farms, particularly the microbiological quality, should be of concern.

To ensure the safety and satisfactoriness of milk products, the physical and microbiological parameters of raw milk are evaluated during milk production. Microorganisms play an important role in the deterioration or spoilage of raw milk and milk products. The level of microbial contamination could indicate the hygienic practices and cleanliness of the environment on the goat farm (Tombarkiewicz *et al*., 2009). The groups of microorganisms commonly used as microbiological indicators in raw milk include total bacteria, coliforms, thermoduric bacteria, proteolytic bacteria and lipolytic bacteria (Quigley *et al*., 2013). Total bacteria mostly refer to mesophilic aerobic bacteria that indicate the hygienic practice of the milking process and transportation. A high level of total bacteria results in a high risk of deterioration of finished milk products (Martin *et al*., 2023). Coliform bacteria are generally found in the environment and faecal materials. Thus, a high concentration of coliforms in raw milk could indicate the presence of faecal pathogenic bacteria (Martin *et al*., 2023). Proteolytic and lipolytic bacteria are groups of bacteria that can produce enzymes, i.e. proteases and lipases, respectively, during their growth. High concentrations of proteolytic or lipolytic enzymes can degrade milk components into small molecules, resulting in off-flavours and denaturation of raw milk and milk products (Qin *et al*., 2023). Contamination with thermoduric bacteria directly affects the shelf life and quality of pasteurised milk because of thier ability to tolerate the temperature present during the pasteurisation process (Gleeson *et al*., 2013).

The direct factors associated with microbial contamination of raw cow's milk are the animal's health status, milking hygiene and storage conditions. However, environmental factors have been reported as indirect factors affecting the microbiological quality of raw milk (Toghdory *et al*., 2022). For dairy farming, weather conditions are considered one of the crucial determinants of animal health and raw milk quality (Sankar, 2023). According to a 12-month study on raw milk in China, the microbial load was reported to be the most diverse in June and the least in December. Temperature and humidity were suggested as the factors associated with variation of the microbiota in milk (Li *et al*., 2018). Similarly, the total bacterial count in raw goat milk increased from May to October in Canada, when high temperatures may affect the growth of microbes in a bulk dairy tank (Tirard-Collet *et al*., 1991).

Nowadays, the demand for goat milk is increasing among Muslims, resulting in the highest density of goat farming in the lower part of Southern Thailand, home to the largest Muslim community in Thailand (Wasiksiri *et al*., 2010; Umar *et al*., 2017). Compared to bovine milk, knowledge of goat milk production in Thailand is still limited, particularly regarding its microbiological quality. Therefore, this study aimed to evaluate the microbiological quality of raw goat milk in the lower part of Southern Thailand and demonstrate the variation in microbiological quality affected by climatic factors.

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Materials and methods

Sampling and data collection

From November 2020 to October 2022, a total of 52 raw goat milk samples were purchased from eight dairy goat farms located in Pattani, Phatthalung, Songkhla and Yala provinces. Participating farms were private smallholder businesses located distantly from living areas. In these farms, the roughage such as grasses and Leucaena leucocephala were primarily used, while concentrated feed was occasionally provided as a supplement. The milk was collected by hand-milking once a day in the morning and kept chilled prior to transportation to customers. Milk samples were collected from bulk tanks after complete milking each day. The samples were taken from milk storage tanks and kept in sterile plastic containers at temperatures below 4°C during transportation. The laboratory process was conducted within 4 hours after sample collection. To study the association between the microbiological quality of goat milk and climatic factors (i.e. rainfall for seven days prior to milk collection (rainfall), relative humidity and ambient temperature), sample collection was conducted periodically throughout the year. Climatic data were obtained from daily records of weather stations in the area nearest the participating goat farms.

Microbiological analyses

Bacterial enumeration was employed to examine the microbiological quality of raw goat milk. In the current study, five microbiological parameters (i.e. total bacteria, coliforms, proteolytic bacteria, lipolytic bacteria and thermoduric bacteria) were included. To determine the estimated bacterial concentration in homogenized milk samples, serial ten-fold dilutions were performed using 0.85% saline solution. Viable plate counts of total bacteria, coliform bacteria, proteolytic bacteria and lipolytic bacteria were applied on plate count agar (BD DifcoTM, USA), violet-red bile agar (BD DifcoTM, USA), plate count agar (BD DifcoTM, USA) with 1% skim milk (HiMedia Laboratories, India) and tributyrin agar (HiMedia Laboratories, India), respectively. Testing for thermoduric bacteria was performed by heating the milk at pasteurization temperature (63°C for 30 minutes) prior to inoculation on plate count agar. For microbiological analyses, samples were incubated aerobically at 37°C for 24–48 hours. All viable bacterial colonies were counted for total bacterial and thermoduric bacterial enumeration. Proteolytic and lipolytic bacteria were characterised by the clear zone surrounding the colonies. Coliform bacteria on violet-red bile agar were differentiated by purple-to-red colonies with or without a precipitation zone. Microbiological parameters were displayed as the number of bacterial colonies per millilitre of milk sample or CFU/mL.

Statistical analyses

The arithmetic mean and standard deviation of the log-transformed data were applied to analyse the microbiological parameters of milk samples. Seasons of microbiological parameters were defined as follows: rainy season (September to December) and dry season (January to August). The criteria for seasonal classification in this study are based on the precipitation levels reported by the Thai Meteorological Department. The levels of microbial contamination of goat milk samples that were collected in the rainy and dry seasons were compared by the Wilcoxon rank-sum test. The linear relationship between microbiological parameters and climatic factors was evaluated by the Pearson product-moment correlation coefficient. The bacterial concentration in milk samples was designated as the dependent variable, whereas climatic factors were assigned as independent variables. A $P<0.05$ was considered the threshold for statistical significance. Statistical analyses and data visualisation for the present study were conducted using RStudio software (RStudio©, PBC, Boston, MA).

Results

Among the five microbiological parameters examined in goat milk, total bacteria showed the highest average concentration (4.21±0.30 log CFU/mL), followed by lipolytic bacteria (3.75±0.30 log CFU/mL), proteolytic bacteria (3.58±0.34 log CFU/mL), coliforms (3.10±0.40 log CFU/mL) and thermoduric bacteria (1.73±0.15 log CFU/mL). The maximum bacterial contamination in goat milk samples in the current study was 7.95 log CFU/mL. A statistical comparison between the microbial loads of the two seasons showed that contamination by total bacteria, lipolytic bacteria, proteolytic bacteria and coliforms in the rainy season was significantly higher than that in the dry season ($P<0.05$) (Fig. 1). By contrast, there was no statistically significant difference between thermoduric bacteria found in milk samples collected in the dry and rainy seasons.

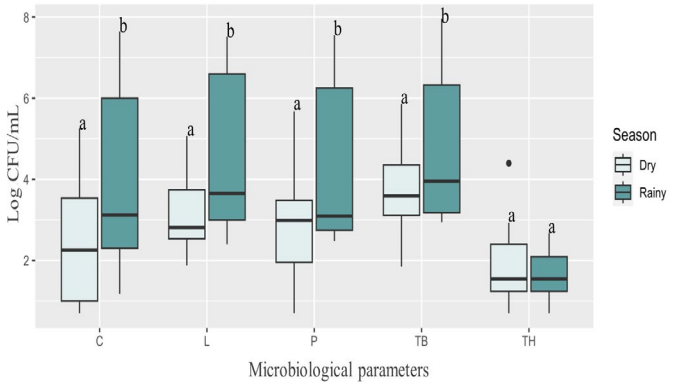

Fig. 1. Overall microbiological contamination (TB: total bacterial count, C: coliform count, P: proteolytic bacterial count, L: lipolytic bacterial count, TH: thermoduric bacterial count) (log CFU/mL) classified by season representing the southernmost part of Thailand. Different letters within the same microbiological parameter refers to a statistically significant difference at a $P<0.05$.

In the current study, Pearson's correlation was used to measure the relationship between microbiological parameters (i.e. total bacteria, coliforms, proteolytic bacteria, lipolytic bacteria and thermoduric bacteria) and climatic factors (i.e. rainfall, relative humidity and ambient temperature), which were demonstrated by correlogram (Fig. 2). Four microbiological parameters i.e. total bacteria, coliforms, proteolytic bacteria and lipolytic bacteria were significantly correlated with rainfall, relative humidity and ambient temperature. Rainfall and relative humidity were positively correlated with total bacteria, coliforms, proteolytic bacteria and lipolytic bacteria, while an inverse correlation between ambient temperature and those four microbiological parameters was described. The correlation coefficient, which measures the degree of the linear relationship between two variables, ranged from –0.6 to 0.57 in the current study. The value of the correlation coefficient indicated that the linear relationships between lipolytic bacteria and three climatic factors were relatively strong compared to other microbiological parameters. The relationships between thermoduric bacteria and three climatic factors (i.e. rainfall, relative humidity and ambient temperature) were not statistically significant.

Discussion

Generally, the quality of a dairy product depends on the extent of microbiological contamination of the raw material. A high microbial load in raw milk is bound to adversely affect subsequent products in terms of their physical and organoleptic properties (Valente *et al.*, 2019). With proper milking hygiene, milk drawn from a healthy animal should have a total bacterial count between 500 and 1,000 CFU/mL (Tegegne and Tesfaye, 2017). However, sterile milk collection is virtually impossible at the farm level. To assure the quality and safety of milk products, the limits of microbiological contamination in raw milk are issued by the national

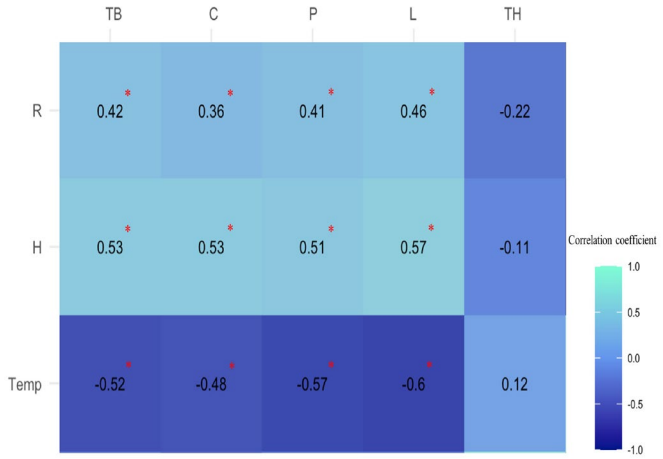

Fig. 2. Pearson's correlation between microbiological parameters (TB: total bacterial count, C: coliform count, P: proteolytic bacterial count, L: lipolytic bacterial count, TH: thermoduric bacterial count) and climatic factors (R: rainfall in the seven days prior to milk collection, H: humidity, Temp: ambient temperature). Correlation coefficients for all pairs of variables were described. Asterisks represent statistically significant correlations between two variables (P<0.05).

institutes of each country. In Thailand, the National Bureau of Agricultural Commodities and Food Standards, Ministry of Agriculture and Cooperatives (2008) has defined the level of microbiological contamination that is allowed to be present in raw goat milk, i.e. the total bacterial count, coliforms and heat-resistant bacteria (National Bureau of Agricultural Commodity and Food Standards 2008). In addition, other microbes that cause the deterioration of raw and finished dairy products, such as lipolytic and proteolytic bacteria, can be further defined by their characteristics (Baur *et al*., 2015). In the current study, the average bacterial concentration of microbiological parameters in goat milk samples did not exceed the limit defined by Thai agricultural standards. Moreover, the microbiological performance of goat milk in the current study seemed to be favourable in the dry season. According to a study in Ethiopia, a statistically significant increase in total aerobic mesophilic bacteria and coliforms was reported in the dry season, whereas the *Escherichia coli* count in the dry season was lower than that in the wet season (Nahusenay *et al*., 2023). Interestingly, a study in Hungary found that the mean total plate count, total coliform count and *Staphylococcus aureus* count in raw milk were higher in the summer period (Petróczki *et al*., 2020). The level of bacterial contamination in raw milk can be associated with hygienic practices on the farm, the geographical location, the cleanliness of the dairy farm's environment and climatic factors (Zweifel *et al*., 2005; Lopes *et al*., 2021).

Overgrowth of microorganisms in raw milk results in various changes in the properties of milk products, depending on the type of microbes (Yuan *et al*., 2022). The total number of bacteria is the most common indicator of the microbiological quality of raw milk (Wang *et al*., 2023). High total bacteria contamination in raw milk affects the taste and safety of dairy products (Washaya *et al*., 2022). In addition, the coliform bacterial count is another microbiological parameter commonly used in the food industry as a hygienic indicator. A high level of milk contamination by coliform bacteria indicates unsanitary practices during the milking process, improper milk storage or milk drawn from mastitis animals (Martin *et al*., 2016). Normally, pasteurisation or other heat treatment methods used on raw milk can destroy most of the viable microorganisms that cause milk spoilage (Dash *et al*., 2022). Thermoduric or heat-resistant bacteria are troublesome for the dairy industry because of their ability to survive pasteurisation. Thus, the presence of these bacteria can affect the shelf-life of dairy products (Júnior *et al*., 2018). Moreover, certain bacteria (e.g. lipolytic and proteolytic bacteria) contaminating raw milk can produce enzymes that degrade milk components even if the organism has been destroyed (Yuan *et al*., 2018).

According to previous studies, relative humidity, ambient temperature and precipitation promote the survival of organisms in the environment (Feliciano *et al*., 2020). Thus, dairy goats in Thailand, which are commonly exposed to the surrounding environment (Raksasiri *et al*., 2022), can be easily affected by changes in the weather. In the current study, the positive correlation between two climatic factors (i.e. rainfall and relative humidity) and four microbiological parameters (i.e. total bacteria, coliforms, proteolytic bacteria and lipolytic bacteria) were reported. On the other hand, we found a negative correlation between ambient temperature and those four microbiological parameters. In the northeastern region of Iran, Toghdory *et al*. (2022) found that an increase in temperature resulted in a 13.7% higher microbial load in cow's milk. Meanwhile, a negative correlation between humidity and the microbial count in cow's milk was described. According to previous studies, increasing temperature was associated with economic losses due to milk spoilage (Montcho *et al*., 2021). Djekic *et al*. (2020) reported that high accumulated precipitation was associated with an increase in the total plate count in raw milk collected in Serbia. The lack of correlation between thermoduric bacteria and all climatic factors reported in the current study indicated that contamination of raw goat milk with thermoduric bacteria might be affected by unknown factors and needs further investigation.

In the current study, microbiological contamination in raw goat milk did not considerably exceed the national standards of Thailand. However, the presence of certain types of bacteria (such as lipolytic, proteolytic and thermoduric bacteria) can influence the quality of pasteurized milk products. In addition, small-scale goat milk producers in the southernmost part of Thailand should be particularly aware of the microbiological quality of raw milk during the rainy season when a high humidity level is inevitable. To keep satisfactory quality of goat milk during high rainfall and high relative humidity period, air ventilation in the dairy farm environment, hygienic milking practices and farm biosecurity should be strictly performed.

Conclusion

The current study demonstrated the level of microbiological contamination in goat milk and seasonal effect on its quality. Our analysis revealed that climatic factors (rainfall, relative humidity and ambient temperature) were significantly correlated with certain microbiological parameters. For the further study, diversified milk samples should be included to strengthen the accuracy of the analysis.

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Conflict of interest

The authors have no conflict of interest to declare.

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