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Development of a Functional Goat Milk Yogurt Fortified with Canine-Derived Probiotics: Physicochemical Properties, Gastrointestinal Survival, and Palatability in Dogs

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Abstract

Dog yogurt products have gained increasing popularity among pet owners because of their nutritional value and their potential as vehicles for delivering probiotics to companion animals. This study aimed to develop a host-specific probiotic goat milk yogurt for dogs by combining canine-derived probiotic strains with selected yogurt starter cultures isolated from commercial yogurts. Six lactic acid bacteria (LAB) isolates were obtained from ten commercial yogurt products and identified as *Lactocaseibacillus paracasei* subsp. *paracasei*, *Limosilactobacillus fermentum*, and *Streptococcus salivarius* subsp. *thermophilus* using the API 50 CHL system. Among the isolates, *Lactocaseibacillus (Lac.) paracasei* LPC1 and *Streptococcus (S.) thermophilus* ST8 were selected as starter cultures based on their technological properties. Functional goat milk yogurts were produced by co-culturing the selected starter cultures with canine-derived probiotics, namely *Agrilactobacillus fermenti* Pom1, *Limosilactobacillus fermentum* Pom5, *Pediococcus pentosaceus* Chi8, or their cocktail. Compared with probiotic monocultures, the incorporation of *Lac. paracasei* LPC1 and *S. thermophilus* ST8 significantly improved yogurt structure by reducing syneresis and increasing the water-holding capacity. During 7 days of storage at 4°C, all formulations maintained stable physicochemical properties, with pH values ranging from 4.1 to 4.4 and titratable acidity from 0.77% to 1.13%. Viable LAB populations remained within 10⁷–10⁸ CFU/g throughout storage. Simulated gastrointestinal assays showed that substantial populations of viable LAB and *S. thermophilus* survived exposure to gastric and intestinal conditions, indicating a protective effect of the yogurt matrix. Palatability testing in 30 dogs demonstrated excellent acceptance of all formulations, with complete consumption rates of 96.7–100.0% and average consumption times of 28–38 s. Overall, co-culturing *Lac. paracasei* LPC1 and *S. thermophilus* ST8 with canine-derived probiotics successfully produced a functional goat milk yogurt with desirable physicochemical properties, high palatability, and sustained LAB viability during refrigerated storage, supporting its potential as a probiotic delivery vehicle for dogs.

Keywords: canine probiotics, *Agrilactobacillus fermenti*, *Limosilactobacillus fermentum*, *Pediococcus pentosaceus*, goat milk yogurt, simulated gastrointestinal survival

1. Introduction

Yogurt is one of the most widely consumed fermented dairy products worldwide and is traditionally produced by fermenting milk with the starter cultures *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Rashwan et al., 2022; Wijesekara et al., 2022). During fermentation, these bacteria metabolize lactose into lactic acid, acetaldehyde, and other metabolites that contribute to the characteristic flavor, aroma, texture, and nutritional quality of yogurt (Liu et al., 2022; Dan et al., 2023). Collectively, this microbial community drives carbohydrate fermentation through the generation of organic acids while simultaneously improving the absorption and bioavailability of minerals. Additionally, these microbes strengthen the epithelial barrier and regulate metabolic and immune pathways related to infection, inflammation, and lipid homeostasis (Latif et al., 2023). Consequently, the identification of lactic acid bacteria (LAB) strains that possess both desirable technological characteristics and functional probiotic properties has become a major focus in the development of functional fermented dairy products (El Kahlout et al., 2018).

Growing awareness of companion animal health has accelerated the development of functional pet foods enriched with probiotics. In dogs, probiotic supplementation has been associated with improved gastrointestinal health, modulation of the intestinal microbiota, enhancement of immune responses, and reduced susceptibility to enteric pathogens (Schmitz & Suchodolski, 2016; Grześkowiak et al., 2015). As in humans, dogs may benefit from the regular consumption of probiotic-containing fermented dairy products when administered at appropriate levels (Pilla & Suchodolski, 2020). Accordingly, probiotic yogurt represents a promising vehicle for delivering beneficial microorganisms to the canine gastrointestinal tract while providing a nutritious and highly palatable dietary supplement.

Host-specific probiotics are particularly advantageous because of their superior adaptation to the gastrointestinal environment of the target host, which enhances their colonization, persistence, and functional activity compared with non-host-derived strains (Han et al., 2021). Previous research has demonstrated that canine-derived LAB can effectively colonize the intestinal tract, inhibit enteric pathogens such as *Salmonella* spp. and *Clostridium perfringens*, alleviate intestinal inflammation, and

modulate host immune function (Lone et al., 2021; Jiang et al., 2024).

To establish a strong scientific basis for developing a host-specific canine functional food, three probiotic strains with commercial potential *AgriLactobacillus fermenti* Pom1, *Limosilactobacillus fermentum* Pom5, and *Pediococcus pentosaceus* Chi8 were selected from our repository for further development. In our previous work, these canine-derived strains were comprehensively characterized and exhibited exceptional functional probiotic properties. These included robust tolerance to simulated gastrointestinal conditions, favorable safety profiles confirmed by both phenotypic and whole-genome analyses, and functional beta-galactosidase activity for enhanced lactose utilization (Foongsawat et al., 2023a; Sunthornthummas et al., 2025). Furthermore, these strains demonstrated exceptional cell-surface hydrophobicity, a strong auto-aggregation ability, high free-radical-scavenging (DPPH) antioxidant activity, and excellent adhesion to Caco-2 epithelial cells (ranging from 87.63% to 95.08%). These specific strains were selected as ideal candidates because their robust acid tolerance and superior epithelial adhesion suggest a strong potential for post-ingestion intestinal colonization, while their beta-galactosidase activity offers a targeted mechanism to mitigate the lactose intolerance commonly observed in companion animals. To further improve their technological applicability, we subsequently developed an alginate–goat milk-based microencapsulation system, which significantly enhanced probiotic survival during refrigerated storage, pasteurization, and simulated gastrointestinal transit compared with free cells (Foongsawat et al., 2023b). More recently, this platform was further optimized by incorporating β -glucan into the alginate–goat milk matrix, resulting in high encapsulation efficiencies (88.25–99.33%) and significantly improved survival of *A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8 following freeze-drying, two months of storage, and simulated gastrointestinal exposure (Pringsulaka et al., 2025). These findings provide strong evidence that the selected canine-specific probiotic strains possess not only desirable biological functions but also the technological robustness required for incorporation into stable functional food formulations.

Despite these promising probiotic attributes, successful application in a functional food requires more than demonstrating *in vitro* probiotic properties.

The selected strains must also be technologically compatible with yogurt manufacture, remain viable during refrigerated storage, survive passage through the gastrointestinal tract after incorporation into the food matrix, and produce a product with acceptable physicochemical characteristics and palatability (Dave & Shah, 1997). To date, these technological and functional characteristics have not been investigated for canine-derived probiotic strains incorporated into fermented dairy products.

Goat milk was selected as the substrate for yogurt production because of its favorable nutritional composition and digestibility compared with cow milk. Although the two milks have similar macronutrient contents, goat milk contains smaller fat globules, higher proportions of medium-chain fatty acids and beta-casein, lower lactose concentrations, and relatively higher levels of naturally occurring oligosaccharides (Park et al., 2007; Nayik et al., 2022; Paszczyk et al., 2023). From a nutritional standpoint, these inherent compositional traits contribute to improved digestibility and provide a highly compatible matrix for supporting the survival of beneficial microorganisms (Lima et al., 2018). Consequently, goat milk represents an attractive matrix for developing probiotic fermented products intended for canine consumption.

Despite the increasing availability of probiotic products for humans and livestock, studies investigating fermented dairy products containing host-specific canine probiotics remain very limited. To the best of our knowledge, this is the first study to develop a goat milk yogurt supplemented with probiotic strains isolated from dogs and to evaluate the ability of these probiotics to maintain high viability and to survive within the final yogurt matrix. Furthermore, no previous study has comprehensively evaluated the technological performance of these canine-derived probiotics during yogurt manufacture, their viability throughout refrigerated storage, their survival under simulated gastrointestinal conditions after incorporation into a yogurt matrix, and the palatability of the resulting product in dogs. These evaluations are essential because maintaining high viable probiotic populations during storage and gastrointestinal transit is a prerequisite for successful delivery to the intestinal tract and for the potential modulation of the canine gut microbiota and associated health benefits.

2. Objectives

This study aimed to develop a host-specific probiotic goat milk yogurt supplemented with canine-derived probiotic strains and to characterize its physicochemical, functional probiotic, and organoleptic properties. We evaluated fermentation performance, viability during refrigerated storage, and survival under simulated canine gastrointestinal conditions in order to verify the capacity of the strains to survive transit intact—a critical prerequisite for downstream intestinal colonization. Finally, the product's palatability was evaluated in dogs. Collectively, these parameters establish the essential *in vitro* baseline required to justify future *in vivo* gut microbiota and immunomodulatory efficacy trials in canine nutrition.

3. Materials and methods

3.1 Isolation and Identification of Lactic Acid Bacteria (LAB) from Commercial Yogurt

Commercial yogurt samples were serially diluted in sterile peptone water and spread onto de Man, Rogosa and Sharpe (MRS) agar (Himedia, India) and M17 agar (Himedia, India), both supplemented with 0.3% (w/v) CaCO_3 . The plates were incubated microaerophilically at 37°C for 48–72 h. Colonies exhibiting clear surrounding zones were selected and purified by repeated streaking on the same media. Pure isolates were preliminarily characterized based on colony morphology, Gram staining, and catalase activity. Gram-positive and catalase-negative isolates were further identified using the API 50 CHL system (bioMérieux, France) according to the manufacturer's instructions.

3.2 Selection of Yogurt Starter Cultures

Presumptive *Lactobacillus* and *Streptococcus* isolates obtained from Section 3.1 were individually cultured to achieve high initial cell concentrations of approximately 8.0–9.0 log CFU/mL (greater than 10^8 CFU/mL). Five milliliters of each standardized culture were inoculated into 30 mL of pasteurized goat milk in sterile containers (achieving an initial inoculation density of approximately 7.0–8.0 log CFU/mL within the milk matrix). The mixtures were incubated under microaerophilic conditions at 37°C for 24 h, at which point visual coagulation occurred and a target pH of approximately 4.5 was reached. The resulting yogurt samples were immediately evaluated based on total viable cell counts, pH, titratable acidity, syneresis, and water-holding capacity (WHC) to

select the optimal starter pair. Following these treatments, the viability and retention of the selected strains were monitored closely to ensure accurate strain tracking throughout the study.

3.2.1 Enumeration of LAB

Ten grams of yogurt were aseptically transferred into a sterile stomacher bag containing 90 mL of 0.1% peptone water and homogenized for 2 min using a stomacher (Stomacher 400 Circulator, Seward Medical Ltd., London, UK). Serial decimal dilutions were prepared in 0.1% peptone water. Total lactobacilli counts (TLC) were determined on MRS agar, whereas total *Streptococcus* counts (TSC) were determined on M17 agar. Plates were incubated at 37°C for 48–72 h under microaerophilic conditions, and the results were expressed as colony-forming units per gram (CFU/g) (Da Silva et al., 2018).

3.2.2 Determination of pH and Titratable Acidity

The pH of the yogurt samples was measured using a calibrated digital pH meter. Titratable acidity (TA) was determined according to the method described by Oladipo et al. (2014). Briefly, 10 g of yogurt were mixed with 10 mL of distilled water, followed by the addition of two drops of 1% phenolphthalein indicator. The mixture was then titrated against 0.1 N NaOH until a faint, persistent pink endpoint was reached. The results were expressed as the percentage of lactic acid equivalent (% lactic acid).

3.2.3 Syneresis Measurement

Syneresis was determined according to the method of Varelziz et al. (2016). Ten grams of yogurt sample were centrifuged at 5,000 rpm for 10 min at 4°C. The released whey (supernatant) was carefully separated and weighed. Syneresis was calculated using the following equation:

$$\text{Syneresis (\%)} = (\text{Weight of released whey} / \text{Weight of yogurt sample}) \times 100$$

3.2.4 Water-Holding Capacity (WHC)

WHC was determined according to the procedure of Luana et al. (2014). Yogurt samples (10 g) were centrifuged at 5,000 rpm for 10 min at 20°C. The supernatant was removed and weighed, and the WHC was calculated as follows:

$$\text{WHC (\%)} = [(\text{Weight of yogurt} - \text{Weight of supernatant}) / \text{Weight of yogurt}] \times 100$$

3.3 Production of Probiotic Goat Milk Yogurt

The starter culture combination demonstrating the most desirable physicochemical and technological characteristics (*Lac. paracasei* LPC1 and *S. thermophilus* ST8) was selected for probiotic yogurt manufacture. Three canine-derived probiotic strains—*Agrilactobacillus (A.) fermenti* Pom1, *Limosilactobacillus (Lim.) fermentum* Pom5, and *Pediococcus (P.) pentosaceus* Chi8—were incorporated individually alongside the selected starter culture pair. For each formulation, the respective probiotic strain and the starter cultures were inoculated at approximately 10^8 each (5 mL of each component culture, maintaining a 1:1:1 ratio) into a tightly closed sterile bottle containing 30 mL of pasteurized goat milk and incubated at 37°C for 24 h (until complete coagulation and target baseline pH were achieved). Following fermentation, the yogurt samples were transferred and stored at 4°C for 7 days, during which physicochemical properties, probiotic viability, and total bacterial counts were systematically monitored.

3.4 Palatability Test in Dogs

The palatability trial was performed at Samut Prakan, Thailand, by an accredited contract research organization (The Unique Laboratory Co., Ltd.). The panel consisted of 30 healthy adult dogs, including 18 males and 12 females, representing both small- and large-breed dogs. The dogs ranged in age from 1 year to 5 years 4 months and had a mean body weight of 20.9 ± 10.3 kg (range: 2.0–36.7 kg). Dogs were housed individually in indoor kennel compartments under a controlled 12-h light/dark cycle at a temperature of $24 \pm 2^\circ\text{C}$ and a relative humidity of $55 \pm 10\%$. Animals were fed a standardized commercial maintenance dry kibble diet once daily, with fresh water available *ad libitum*. Five experimental yogurt formulations were evaluated: three single-strain formulations (each fortified individually with *A. fermenti* Pom1, *Lim. fermentum* Pom5, or *P. pentosaceus* Chi8), one multi-strain cocktail formulation (incorporating all three canine-derived probiotic strains), and one baseline control yogurt (fermented solely with the starter cultures *Lac. paracasei* LPC1 and *S. thermophilus* ST8). Palatability was assessed using a single-bowl total acceptance test to verify the absolute consumption and market feasibility of each specific product variation. A threshold of complete consumption within a fixed feeding window was used to confirm that neither the individual probiotic strains nor their multi-strain

combination adversely altered palatability compared with the baseline control matrix.

The experimental protocol and animal handling procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of ANLAR Service Co., Ltd. (Approval No. IACUC-ANLAR-2026/009). The animal study was conducted at The Unique Laboratory Co., Ltd., Samut Prakan, Thailand. All experimental procedures were conducted in accordance with internationally accepted guidelines for the ethical care and use of animals in research.

3.5 Survival under Simulated Gastrointestinal Conditions

3.5.1 Simulated Gastric Fluid (SGF) Tolerance

Gastric survival of the probiotic strains was assessed according to the methodology described by Ranadheera et al. (2012). Simulated gastric fluid (SGF) was prepared by dissolving pepsin (3.2 g/L; Sigma-Aldrich, USA) in a sterile saline electrolyte solution (0.2% w/v NaCl). The final pH of the SGF was adjusted to 2.0 using 1 M HCl. One gram of the probiotic yogurt sample was uniformly suspended in 9 mL of the prepared SGF (pH 2.0) and incubated at 37°C under continuous agitation at 100 rpm for 3 h. Viable probiotic cell counts were enumerated at 1, 30, 60, and 180 min to evaluate survival during simulated gastric transit.

3.5.2 Simulated Intestinal Fluid (SIF) Tolerance

Simulated intestinal fluid (SIF) was formulated by dissolving pancreatin (1.0 g/L; Sigma-Aldrich) and purified bile salts (0.3% w/v; Himedia, India) in a sterile 0.05 M potassium phosphate buffer solution (KH₂PO₄), with the final pH adjusted to 8.0 using 1 M NaOH. One gram of the yogurt sample was thoroughly mixed with 9 mL SIF (pH 8.0). The mixture was subsequently incubated at 37°C with continuous shaking at 100 rpm for 4 h. Viable probiotic counts were monitored and enumerated at 1, 120, and 240 min. All gastrointestinal simulation assays were performed in triplicate to ensure reproducibility.

3.6 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (Version 28.0.1.0, IBM Corp., Armonk, NY, USA). Data are presented as mean $\pm$ standard deviation (SD) of triplicate determinations. Data normality and variance homogeneity were

confirmed using Shapiro-Wilk and Levene's tests, respectively. Statistically significant differences among the mean values of the experimental treatments were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test for multiple pairwise comparisons. Statistical significance was defined at $p < 0.05$.

4. Results

4.1 Isolation and Identification of LAB from Commercial Yogurt

Lactic acid bacteria were isolated from 10 commercial yogurt samples. Primary selection was based on the formation of a clear zone on MRS agar supplemented with 0.3% (w/v) calcium carbonate, as well as growth on M17 agar. Multiple colonies showing growth on both media were selected as single colonies and subjected to Gram staining in order to examine their morphology and Gram reaction, specifically targeting Gram-positive rods or cocci presumed to be *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Schillinger & Lücke, 1987). Based on morphological characteristics, a total of six isolates were successfully obtained, designated as isolates LPC1, LF3(A), LF3(B), ST8, ST10(A), and ST10(B). As expected for LAB, all six isolates tested negative for catalase, showing no gas bubble formation. The six isolated LAB strains were further characterized using the API 50 CHL standardized biochemical system to evaluate their fermentation profiles across 50 different carbohydrates. Species-level identification was determined using the apiweb™ database software (Table 1). Isolate LPC1 was identified as *Lactocaseibacillus paracasei* ssp. *paracasei* (99.1% ID); isolate LF3(A) as *Limosilactobacillus fermentum* (99.1% ID); and isolate LF3(B) as *Limosilactobacillus fermentum* (99.9% ID). Meanwhile, isolates ST8, ST10(A), and ST10(B) were all identified as *Streptococcus salivarius* ssp. *thermophilus*, with identity percentages of 97.1%, 98.2%, and 94.2%, respectively. This identity is further supported by the selective nature of the isolation process, as these strains were recovered from M17 agar, which is specifically formulated as a selective and differential medium to favor the growth and isolation of *Streptococcus* species while suppressing non-target LAB. Given that these strains were isolated from controlled commercial yogurt matrices where *S. thermophilus* serves as a mandatory industrial starter, their species-level designation is contextually

validated, although the inherent limitations of carbohydrate-fermentation patterns for definitive phenotypic discrimination are acknowledged. Interestingly, although the isolation strategy was designed to recover the conventional yogurt starter culture *L. delbrueckii* subsp. *bulgaricus*, no isolates were identified as this species. Instead, *Lac. paracasei* and *Lim. fermentum* were recovered. This outcome may be attributable to the non-specific nature of the phenotypic selection criteria, which favor acid-producing LAB in general rather than any particular species. Furthermore, differences in survival during

refrigerated storage may have allowed more acid-tolerant non-starter LAB to persist at higher populations than *L. delbrueckii* subsp. *bulgaricus* at the time of isolation. In addition, identification based on API 50 CHL profiles may have limited discriminatory power among closely related lactobacilli. Nevertheless, the isolated *Lac. paracasei* strain demonstrated desirable technological properties and compatibility with *S. thermophilus*, supporting its selection for subsequent yogurt fermentation experiments.

Table 1 Phenotypic carbohydrate fermentation profiles (API 50 CHL) of LAB starters isolated from commercial yogurt

Isolate code	Preliminary morphology	Catalase	API 50 CHL species identification	Identity (%)
LPC1	Gram-positive rod	Negative	<i>Lactocaseibacillus paracasei</i> ssp. <i>paracasei</i>	99.1%
LF3(A)	Gram-positive rod	Negative	<i>Limosilactobacillus fermentum</i>	99.1%
LF3(B)	Gram-positive rod	Negative	<i>Limosilactobacillus fermentum</i>	99.9%
ST8	Gram-positive cocci	Negative	<i>Streptococcus salivarius</i> ssp. <i>thermophilus</i>	97.1%
ST10(A)	Gram-positive cocci	Negative	<i>Streptococcus salivarius</i> ssp. <i>thermophilus</i>	98.2%
ST10(B)	Gram-positive cocci	Negative	<i>Streptococcus salivarius</i> ssp. <i>thermophilus</i>	94.2%



Figure 1 Physical appearance of goat milk yogurts fermented by single probiotic strains alone (a) *A. fermenti* Pom1, (b) *Lim. fermentum* Pom5, and (c) *P. pentosaceus* Chi8 and by co-culturing LAB starter cultures (Isolates LPC1 and ST8) with the respective probiotic strains: (d) Isolates LPC1 and ST8 with *A. fermenti* Pom1, (e) Isolates LPC1 and ST8 with *Lim. fermentum* Pom5, and (f) Isolates LPC1 and ST8 with *P. pentosaceus* Chi8

4.2 Technological Compatibility and Co-culture Yogurt Gelation

Yogurt production was initially evaluated using individual probiotic strains, namely *A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8. After fermentation at 37°C for 24 h, none of the three probiotic strains used alone was able to coagulate goat milk into a yogurt curd with an acceptable texture (Figure 1a–1c). Subsequently, the lactobacilli isolates (LPC1, LF3(A), and LF3(B)) and the *S. thermophilus* isolates (ST8, ST10(A), and ST10(B)) were evaluated. Among these, the combination of starter isolates LPC1 and ST8 demonstrated the most favorable yogurt texture, exhibiting minimal whey separation and superior gel stability.

Goat milk yogurt was subsequently produced by co-culturing the selected LAB isolates (LPC1 and ST8) with the probiotic strains *A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8. In contrast to the single-strain fermentations, this co-culture approach successfully coagulated the goat milk, resulting in a markedly improved curd texture (Figure 1d–1f).

4.3 Physicochemical and Structural Evaluation

Syneresis and water-holding capacity (WHC) are critical physical quality parameters that determine the microstructure, texture, and consumer acceptance of fermented milk products (Sodini et al., 2004). Syneresis refers to the undesirable separation of whey from the continuous protein gel network, typically resulting from structural instability and excessive rearrangement of casein micelles (Lee & Lucey, 2010). Conversely, WHC reflects the ability of the multi-dimensional yogurt matrix to physically entrap and chemically bind the aqueous phase within its pores (Lucey, 2002). In premium-quality yogurt, minimizing syneresis and maintaining a high WHC are essential not only for preventing phase separation during transport and storage but also for preserving a smooth, uniform, and appealing mouthfeel (Lee & Lucey, 2010).

Prior to the integration of probiotics, the screening of starter cultures revealed that the combination of isolates LPC1 and ST8 achieved optimal physical properties, yielding the lowest baseline syneresis of $26.41\% \pm 0.22\%$ and the highest %WHC of $73.70\% \pm 0.15\%$. In contrast, when the probiotic strains (*A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8) were used individually, they failed to form a cohesive gel structure.

The physical properties of these single-strain yogurts were systematically evaluated and compared with the LPC1–ST8 yogurt benchmark (Figure 2). Comparative analysis revealed that the syneresis percentages of the yogurts produced by all three single strains were significantly higher than that of the LPC1–ST8 control. Among these single-strain formulations, *A. fermenti* Pom1 exhibited the highest syneresis ($95.37\% \pm 0.08\%$), followed by *P. pentosaceus* Chi8 ($60.81\% \pm 0.05\%$) and *Lim. fermentum* Pom5 ($54.91\% \pm 0.07\%$). Conversely, the %WHC of all single-strain probiotic yogurts was considerably lower than that of the LPC1–ST8 reference. Among the probiotic formulations, *P. pentosaceus* Chi8 exhibited the lowest WHC ($38.58 \pm 0.22\%$), followed by *A. fermenti* Pom1 ($44.73 \pm 0.03\%$) and *Lim. fermentum* Pom5 ($45.17 \pm 0.13\%$). These findings indicate that the three probiotic strains, when used individually, failed to produce yogurt with textural and water-retention properties comparable to those of the LPC1–ST8 reference yogurt.

The effect of co-culturing on the physical properties of the yogurt was then assessed. Statistical analysis revealed that all co-cultured formulations exhibited significantly lower syneresis values and significantly higher water-holding capacity (WHC) than their corresponding probiotic monocultures ($p < 0.05$). Among the co-cultured yogurts, the formulations containing *A. fermenti* Pom1, *Lim. fermentum* Pom5, or *P. pentosaceus* Chi8 did not differ significantly in WHC (55.32 – 55.88% ; $p > 0.05$), as indicated by the same superscript letter. The mixed-probiotic cocktail exhibited a significantly higher WHC (58.70% ; $p < 0.05$) than the individual probiotic co-cultures but remained significantly lower than the starter-culture control (73.70%). Regarding syneresis, the co-culture containing *Lim. fermentum* Pom5 exhibited the lowest value (41.80%), whereas the co-cultures containing *A. fermenti* Pom1 (44.35%) and *P. pentosaceus* Chi8 (44.66%) did not differ significantly from each other ($p > 0.05$). The mixed-probiotic cocktail showed a significantly higher syneresis value (54.25%) than all individual probiotic co-cultures ($p < 0.05$), indicating that although combining the three probiotic strains improved water-holding capacity, it partially compromised gel stability compared with individual probiotic co-cultures. Overall, these findings demonstrate that incorporating canine probiotic strains together with selected starter cultures effectively enhances water retention and improves the physical stability of goat milk yogurt by reducing whey separation and strengthening the protein gel network.

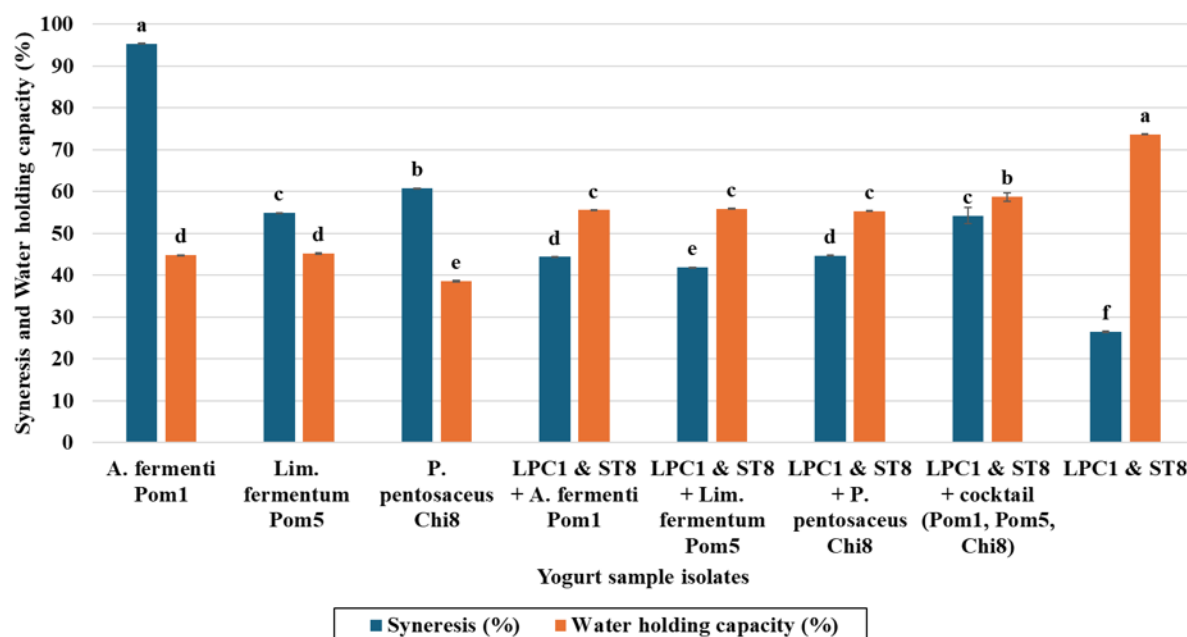


Figure 2 Syneresis (%) and water-holding capacity (WHC, %) of goat milk yogurt formulations produced using different probiotic strains alone and in co-culture with *Lac. paracasei* LPC1 and *S. thermophilus* ST8 during storage.
Note: Data are presented as mean $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences among treatments within the same storage period ($p < 0.05$)

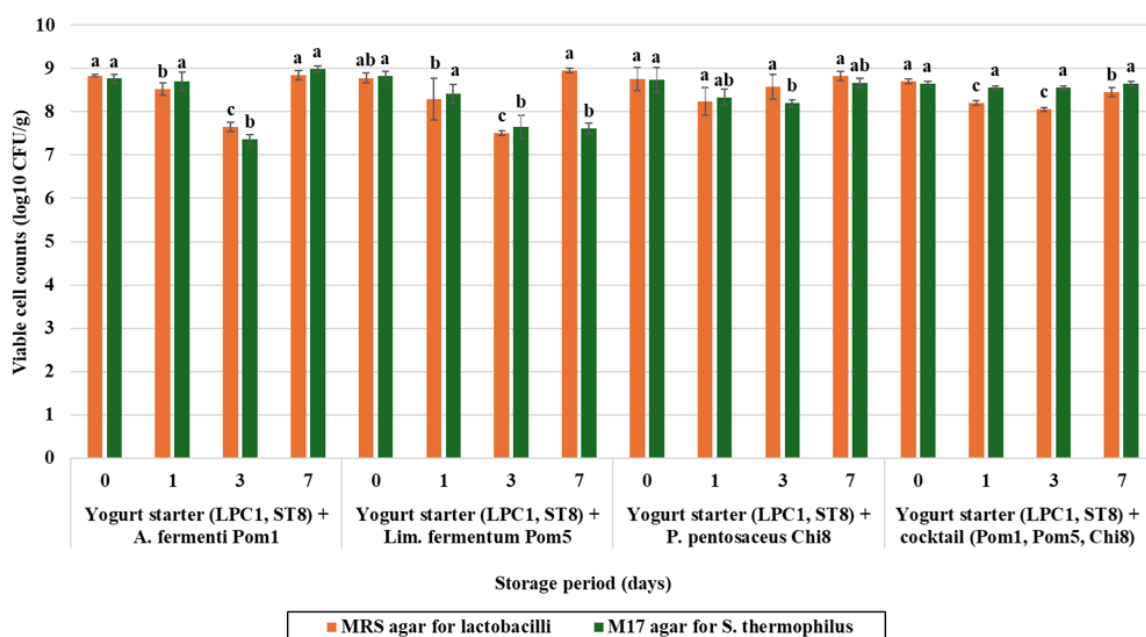


Figure 3 Viable counts of LAB enumerated on MRS agar and *S. thermophilus* enumerated on M17 agar in goat milk yogurt formulations co-cultured with *Lac. paracasei* LPC1, *S. thermophilus* ST8, and canine-derived probiotic strains (*A. fermenti* Pom1, *Lim. fermentum* Pom5, *P. pentosaceus* Chi8, or their cocktail) during storage at 4°C for 7 days. Data are expressed as mean $\pm$ SD ($n = 3$). Different lowercase letters above bars indicate significant differences among storage days within each formulation and microbial group according to Tukey's HSD test following one-way ANOVA ($p < 0.05$)

4.4 Viability and Enumeration of Probiotic Strains in Yogurt

The viability of lactic acid bacteria (LAB) and *S. thermophilus* in the co-cultured yogurt formulations was monitored during refrigerated storage at 4°C for 7 days (Figure 3). Overall, all formulations maintained high microbial populations throughout storage, with viable counts remaining above 10^7 CFU/g and generally ranging between 10^7 and 10^8 CFU/g. For all formulations, both LAB populations enumerated on MRS agar and *S. thermophilus* populations enumerated on M17 agar showed a temporary decline on Day 3, followed by a recovery on Day 7. Significant differences among storage days were observed within each formulation ($p < 0.05$), as indicated by different superscript letters. Among the formulations, yogurts containing *A. fermenti* Pom1 and *Lim. fermentum* Pom5 exhibited the greatest reduction in viable counts on Day 3, whereas formulations containing *P. pentosaceus* Chi8 or the probiotic cocktail maintained comparatively higher bacterial populations during storage. By Day 7, microbial counts in all treatments had recovered to approximately 10^7 – 10^8 CFU/g, demonstrating good survival and stability of both the starter cultures and probiotic-associated LAB under refrigerated conditions. It should be noted that MRS agar supports the growth of both *Lac. paracasei* LPC1 and the probiotic LAB strains; therefore, the counts obtained on MRS agar represent total viable LAB

populations rather than the survival of individual probiotic strains.

4.5 Evaluation of pH and Titratable Acidity (TA)

Changes in pH and titratable acidity (TA) of the co-cultured goat milk yogurts were monitored during refrigerated storage at 4°C for 7 days (Figure 4). All formulations maintained relatively stable pH values throughout storage, ranging from 4.1 to 4.4. Yogurts containing *A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8 generally exhibited lower pH values (4.1–4.2) than the mixed-probiotic formulation (4.37–4.39). Significant differences in pH were observed among storage days within some formulations ($p < 0.05$), although no pronounced decline in pH occurred during refrigerated storage. Similarly, TA values remained relatively stable, ranging from 0.77% to 1.13%, indicating that post-acidification was limited during storage. The highest TA values were observed in yogurts containing *A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8, whereas the mixed-probiotic formulation exhibited slightly lower acidity during the later stages of storage. Overall, the results indicate that the co-culture system comprising the selected starter cultures (*Lac. paracasei* LPC1 and *S. thermophilus* ST8) and canine-derived probiotic strains maintained stable acidification characteristics throughout refrigerated storage, contributing to the physicochemical stability of the yogurt products.

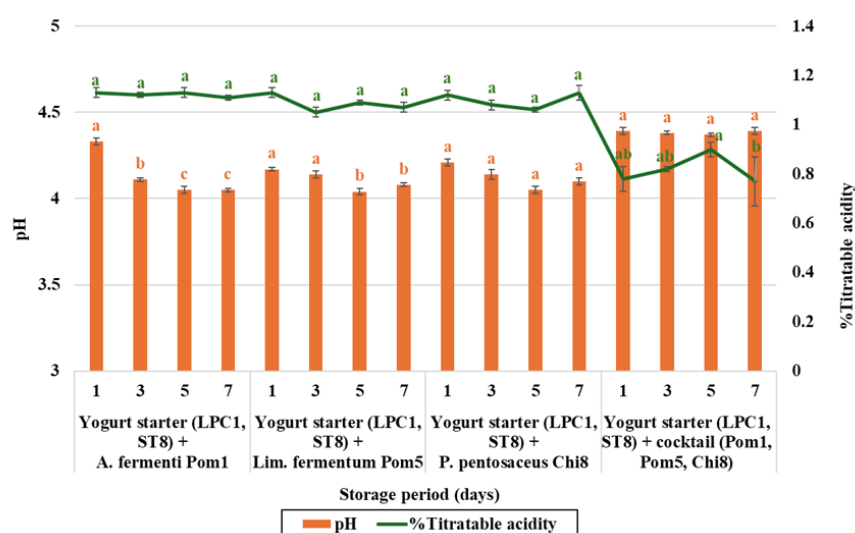


Figure 4 Changes in pH and titratable acidity (TA, % lactic acid) of co-cultured goat milk yogurt formulations containing *Lac. paracasei* LPC1, *S. thermophilus* ST8, and canine-derived probiotic strains (*A. fermenti* Pom1, *Lim. fermentum* Pom5, *P. pentosaceus* Chi8, or their cocktail) during storage at 4°C for 7 days. Data are presented as mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among storage days within each formulation ($p < 0.05$)

4.6 Survival of Probiotics under Simulated Gastrointestinal Conditions

4.6.1 Survival in Simulated Gastric Fluid (SGF)

The survival of LAB and *S. thermophilus* in the co-cultured yogurt formulations was evaluated under simulated gastric conditions for up to 180 min to assess their tolerance to acidic conditions resembling those encountered during gastric transit. Overall, all formulations exhibited a gradual decline in viable cell counts with increasing exposure time; however, substantial populations remained viable throughout the 180-min incubation period (Figure 5). During the initial exposure period (1–30 min), all formulations maintained high viable counts, generally above 10^7 CFU/g. As exposure time increased to 60 and 180 min, a reduction in microbial populations was observed, particularly in the formulations containing *A. fermenti* Pom1 and *P. pentosaceus* Chi8. In

contrast, the formulation containing *Lim. fermentum* Pom5 retained relatively higher viable counts during prolonged exposure, indicating greater tolerance to simulated gastric stress. Notably, the cocktail formulation (Pom1, Pom5, and Chi8) exhibited the greatest overall stability, maintaining viable populations close to their initial levels throughout the incubation period. Despite the observed reductions, all co-cultured formulations retained viable LAB and *S. thermophilus* populations at levels considered sufficient for probiotic delivery after gastric exposure, suggesting that the yogurt matrix provided effective protection against acidic stress. It should be noted that counts obtained on MRS agar represent total viable LAB populations and do not distinguish between *Lac. paracasei* LPC1 and the incorporated probiotic strains.

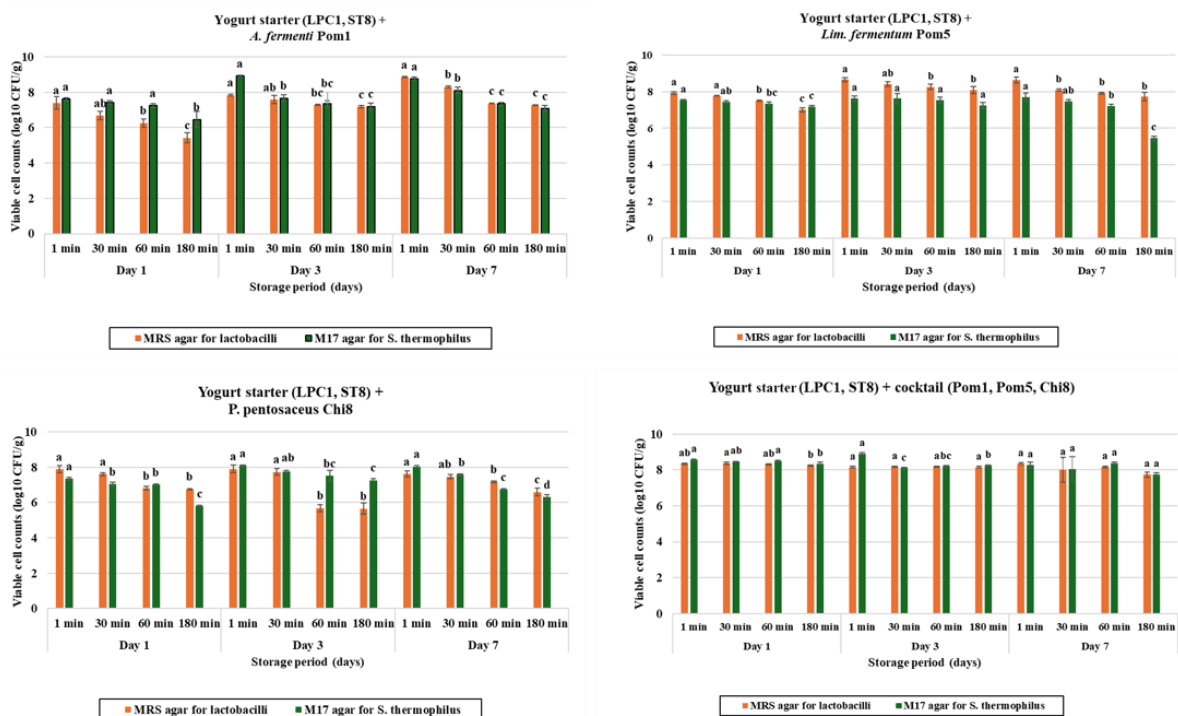


Figure 5 Survival of viable lactic acid bacteria (LAB) enumerated on MRS agar and *S. thermophilus* enumerated on M17 agar in co-cultured goat milk yogurt formulations following exposure to simulated gastric fluid (SGF) for 1, 30, 60, and 180 min after 1, 3, and 7 days of refrigerated storage at 4°C. Yogurt formulations contained *Lac. paracasei* LPC1, *S. thermophilus* ST8, and canine-derived probiotic strains (*A. fermenti* Pom1, *Lim. fermentum* Pom5, *P. pentosaceus* Chi8, or their cocktail). Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among SGF exposure times within the same storage day and formulation according to Tukey's HSD test following one-way ANOVA ($p < 0.05$). Counts obtained on MRS agar represent total viable LAB populations and do not differentiate between LPC1 and the incorporated probiotic strains

4.6.2 Survival in Simulated Intestinal Fluid (SIF)

The survival of LAB and *S. thermophilus* in the co-cultured yogurt formulations was evaluated under simulated intestinal conditions for up to 240 min (Figure 6). Overall, all formulations maintained high viable populations throughout the incubation period, indicating good tolerance to the simulated intestinal environment. The formulations containing *P. pentosaceus* Chi8 and the probiotic cocktail generally exhibited the greatest stability, with viable counts remaining close to their initial levels during the 240-min exposure. In contrast, the formulations containing *A. fermenti* Pom1 and *Lim. fermentum* Pom5 showed more

pronounced reductions in viable counts, particularly after 240 min of incubation. Nevertheless, viable populations in all treatments remained above 10^6 CFU/g and were generally maintained within the range of 10^7 – 10^8 CFU/g throughout the assay. These findings suggest that the yogurt matrix provided effective protection against simulated intestinal stress and supported the persistence of viable LAB and *S. thermophilus* during simulated intestinal transit. It should be noted that counts obtained on MRS agar represent total viable LAB populations and do not distinguish between *Lac. paracasei* LPC1 and the incorporated probiotic strains.

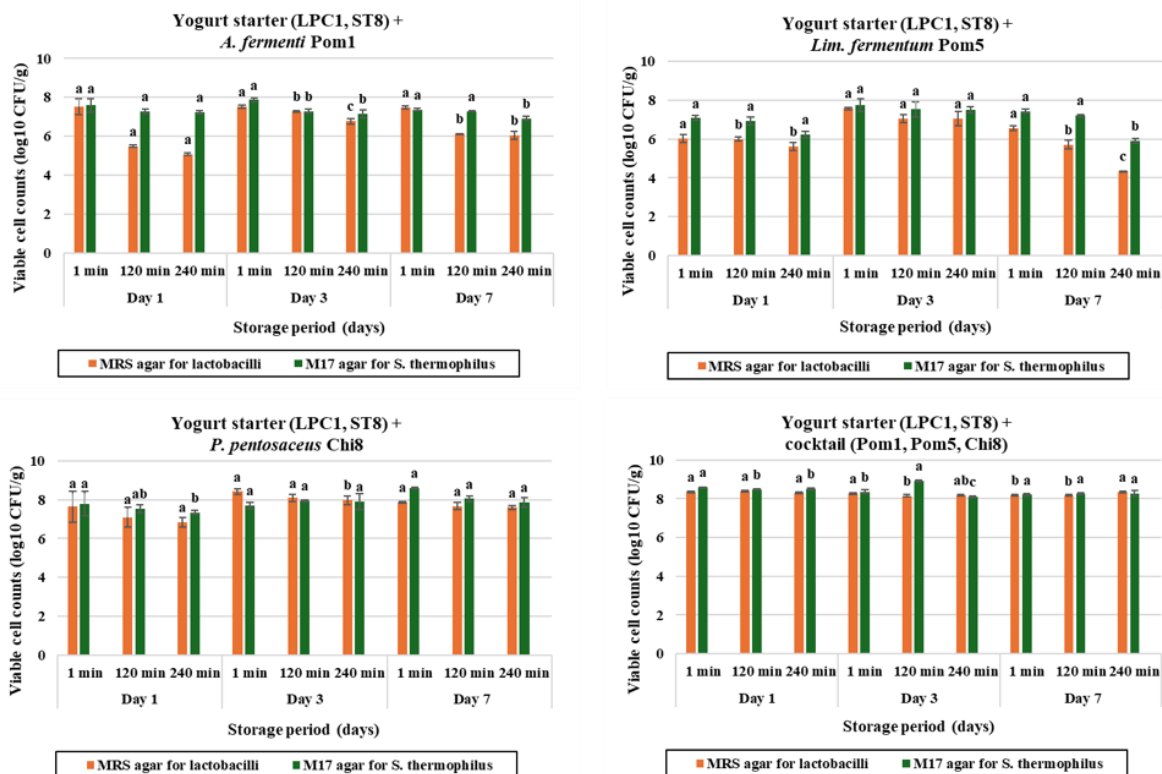


Figure 6 Survival of viable lactic acid bacteria (LAB) enumerated on MRS agar and *S. thermophilus* enumerated on M17 agar in co-cultured goat milk yogurt formulations following exposure to simulated intestinal fluid (SIF) for 1, 120, and 240 min after 1, 3, and 7 days of refrigerated storage at 4°C. Yogurt formulations contained *Lac. paracasei* LPC1, *S. thermophilus* ST8, and canine-derived probiotic strains (*A. fermenti* Pom1, *Lim. fermentum* Pom5, *P. pentosaceus* Chi8, or their cocktail). Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among SIF exposure times within the same storage day and formulation according to Tukey's HSD test following one-way ANOVA (p < 0.05). Counts obtained on MRS agar represent total viable LAB populations and do not differentiate between LPC1 and the incorporated probiotic strains

Table 2 Palatability and consumption metrics of the probiotic yogurt formulations in dogs (n = 30, 2 meals)

Yogurt sample isolates	All sample consumed		Feed Intake		Average meal eating time (sec.)
	Number	%	Gram	%	
LPC1 & ST8	59	98.3	798.0	99.8	28
LPC1 & ST8 + <i>A. fermenti</i> Pom1	58	96.7	797.0	99.6	32
LPC1 & ST8 + <i>Lim. fermentum</i> Pom5	58	96.7	797.0	99.6	38
LPC1 & ST8 + <i>P. pentosaceus</i> Chi8	60	100.0	800.0	100.0	29
LPC1 & ST8 + cocktail (Pom1, Pom5, Chi8)	58	96.7	795.0	99.4	34

4.7 Palatability Test

Palatability evaluation was conducted using a standardized one-bowl test involving 30 healthy dogs over two feeding sessions (60 total choices/meals per formulation) to assess baseline consumption behaviors and product acceptance. To systematically verify whether the fortification of novel canine-derived probiotics altered the sensory profile, the active formulations were evaluated directly against a baseline control yogurt fermented solely with the starter cultures (*Lac. paracasei* LPC1 & *S. thermophilus* ST8). The results, summarized in Table 2, demonstrated exceptional absolute palatability and uniform acceptance across all developed formulations. The baseline control yogurt achieved a high acceptance rate of 98.3% (59/60 meals consumed completely) with a mean intake of 798.0 g (99.8% of feed intake by weight) and a rapid average eating time of 28 seconds. Comparatively, the formulation fortified with *P. pentosaceus* Chi8 achieved a 100% acceptance rate, with all 60 portions completely consumed (800.0 g total intake) within an average eating time of 29 seconds. Formulations containing *A. fermenti* Pom1, *Lim. fermentum* Pom5, and the multi-strain cocktail blend also maintained remarkably high acceptance rates of 96.7%, with total feed intakes reaching 797.0 g, 797.0 g, and 795.0 g (99.4%–99.6% of feed intake by weight), respectively. The average meal eating times for the Pom1, Pom5, and cocktail variations were recorded at 32, 38, and 34 seconds, respectively.

5. Discussion

The growing popularity of probiotics in pet foods and dietary supplements reflects increasing awareness of companion animal health, particularly regarding digestive function and immune support (Schmitz & Suchodolski, 2016; Pilla & Suchodolski, 2020). In the present study, a functional goat milk

yogurt matrix was successfully developed as a specialized dietary delivery vehicle for canine use by integrating host-specific canine-derived probiotic strains (*A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8) with selected starter cultures (*Lac. paracasei* LPC1 and *S. thermophilus* ST8).

A major technological challenge encountered during product development was the inability of the individual probiotic strains to produce an acceptable goat milk yogurt curd when used alone. Traditional yogurt manufacture relies on the cooperative activity of multiple LAB species that establish synergistic metabolic interactions during fermentation (Rashwan et al., 2022; Lee & Lucey, 2010). In conventional yogurt production, *S. thermophilus* rapidly metabolizes lactose and generates metabolites such as formate and carbon dioxide that stimulate the growth of lactobacilli, whereas lactobacilli contribute proteolytic activity and flavor development through peptide and volatile compound production (Lee & Lucey, 2010; Dan et al., 2023). The successful formation of yogurt curd observed in the present study following the incorporation of LPC1 and ST8 highlights the importance of such cooperative interactions in overcoming the technological limitations associated with goat milk fermentation.

Goat milk presents unique challenges for commercial yogurt manufacture because of its characteristically low α_{s1} -casein content and smaller fat globule profile compared with bovine milk, resulting in a weaker and more delicate protein network that is highly susceptible to poor gelation and excessive whey syneresis (Park et al., 2007; Ranadheera et al., 2012). The co-culture system implemented here successfully circumvented these matrix limitations, significantly improving the structural properties of the yogurt matrix as evidenced by reduced syneresis and increased water-holding capacity compared with formulations fermented using

probiotic strains alone. These structural enhancements are primarily attributed to the efficient and accelerated acidification achieved by the co-culture pair, which promotes stronger and more uniform casein network consolidation. The relatively stable pH and titratable acidity observed throughout refrigerated storage further indicate that the co-culture system maintained physicochemical stability without excessive post-acidification.

Maintaining high viable microbial populations throughout storage is a critical requirement for probiotic food products. Although enumeration on MRS agar represented total viable LAB populations rather than individual probiotic strains, all co-cultured formulations consistently maintained LAB counts within the range of 10^7 – 10^8 CFU/g during refrigerated storage. These levels exceed the minimum viable counts generally recommended for probiotic efficacy at the time of consumption (Da Silva et al., 2018; Ranadheera et al., 2012). Refrigeration at 4°C likely contributed to preserving microbial viability by reducing metabolic activity and cellular stress. The transient decline in viable counts observed on Day 3, followed by stabilization or partial recovery by Day 7 (Figure 3), may reflect a temporary physiological adaptation of the bacteria to refrigerated storage and the acidic post-fermentation environment, as previously reported for lactic acid bacteria (Papadimitriou et al., 2016). The simulated gastrointestinal assays further demonstrated the protective effect of the yogurt matrix. Although reductions in viable counts were observed under both simulated gastric and intestinal conditions, substantial populations of LAB and *S. thermophilus* remained viable throughout the exposure periods. The formulations containing *P. pentosaceus* Chi8 and the probiotic cocktail generally exhibited greater stability during simulated intestinal exposure, whereas all formulations retained viable populations above levels commonly regarded as beneficial for probiotic delivery. This degree of structural protection aligns with observations in bovine and caprine probiotic delivery models, where the milk protein–fat matrix functions as a natural mechanical shield that buffers the enclosed cells against enzymatic and low-pH stress (Ranadheera et al., 2012; Dave & Shah, 1997). These findings suggest that the co-cultured yogurt matrix may provide protection against gastrointestinal stress and enhance the likelihood of viable microorganisms reaching the intestinal tract.

The ability of probiotic microorganisms to survive gastrointestinal transit is considered an

important prerequisite for exerting beneficial effects within the host. Surviving populations may contribute to pathogen exclusion, modulation of mucosal immune responses, maintenance of intestinal barrier integrity, and support of gastrointestinal homeostasis (Schmitz & Suchodolski, 2016; Pilla & Suchodolski, 2020). Nevertheless, additional in vivo studies are required to confirm the health-promoting effects of these canine-derived probiotic formulations and to determine their potential roles in specific clinical conditions.

Finally, product acceptance is a critical factor for the success of veterinary functional foods. The one-bowl total acceptance test demonstrated that incorporation of canine-derived probiotics did not adversely affect product acceptance compared with the starter-culture control. Because this assay evaluates absolute acceptance rather than relative preference among formulations, additional two-bowl preference tests would be required to determine the relative preferences of dogs among the probiotic formulations. Nevertheless, the near-complete consumption of all formulations within a short feeding period indicates that the developed yogurts were readily accepted by the dogs, supporting their potential as practical probiotic delivery vehicles for companion animals.

6. Conclusion

This study successfully developed a highly palatable functional goat milk yogurt with improved structural stability for canine application through the co-cultivation of host-specific canine-derived probiotics (*A. fermenti* Pom1, *Lim. fermentum* Pom5, and *P. pentosaceus* Chi8) with the starter cultures *Lac. paracasei* LPC1 and *S. thermophilus* ST8. Although the individual probiotic strains alone were unable to produce an acceptable yogurt curd, the co-culture system effectively improved gel formation and enhanced the physicochemical properties of the final product. The resulting yogurts exhibited reduced syneresis, improved water-holding capacity, and stable pH and titratable acidity, while maintaining high viable LAB populations within the 10^7 – 10^8 CFU/g range throughout 7 days of refrigerated storage. Furthermore, the co-cultured formulations retained substantial viable microbial populations following exposure to simulated gastric and intestinal conditions, indicating that the dairy matrix provided a protective buffering effect against gastrointestinal stress. Palatability testing demonstrated excellent absolute acceptance by dogs, with near-complete

consumption across all tested formulations. Collectively, these findings indicate that a tailored goat milk yogurt matrix represents a promising delivery vehicle for canine-derived probiotic microorganisms and may serve as a practical functional food for companion animals. Studies currently under way in our laboratory are focusing on whole-genome sequencing for definitive strain-level characterization and conducting *in vivo* feeding trials to comprehensively evaluate the modulation of the canine gut microbiota, thereby further confirming the practical efficacy and functional performance of these functional yogurt formulations in target animals.

7. Acknowledgements

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8. Abbreviations

Abbreviation	Full Term
ANOVA	Analysis of variance
API 50 CHL	Analytical profile index 50 carbohydrate <i>Lactobacillus</i> system
CFU	Colony-forming units
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GI	Gastrointestinal
HSD	Honestly significant difference
IACUC	Institutional Animal Care and Use Committee
LAB	Lactic acid bacteria
MRS	de Man, Rogosa and Sharpe
SD	Standard deviation
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
TA	Titrateable acidity
TLC	Total lactobacilli count
TSC	Total <i>Streptococcus</i> count
WHC	Water-holding capacity

9. CRediT Statement

Onanong Pringsulaka: Conceptualization, Methodology, Investigation, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing.

Kamolwan Boonsuya: Methodology, Investigation

Tongdee Munghang: Methodology, Investigation

Kasama Watlom: Methodology, Investigation

Bunyaporn Jintakanont: Methodology, Investigation

Sirinthorn Sunthornthummas: Investigation, Validation

Siriruk Sarawaneeyaruk: Investigation, Validation

Acharya Rangsiruji: Methodology, Validation

Natthida Sudyong: Data curation

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