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Effect of lactic acid bacteria isolated from raw and fermented camel milk on chemical composition and nutrient digestibility of sugarcane bagasse *in vitro*

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ABSTRACT. The purpose of this study was the isolation of the lactic acid bacteria from camel milk and its use in processing of sugarcane bagasse (SB). For isolation and purification of lactic acid bacteria, the raw and fermented camel milk samples were cultured on De Man–Rogosa–Sharpe agar (MRS) culture, and after incubation, the colonies were cultured on liquid MRS culture and again on MRS agar. After gram staining, bacterial DNA extraction and 16S rRNA genome sequencing were performed. The isolated bacterial culture grown in liquid MRS medium was utilized to process SB as described below: SB with no processing (control), SB processed with lactic acid bacteria isolated form fermented camel milk (FCM), (SB-FCM) and SB processed with lactic acid bacteria isolated form raw camel milk (RCM), (SB-RCM). Results indicated that bacteria isolated from RCM and FCM were 99.33 and 99.42% similar to *Enterococcus durans* and *Limosilactobacillus reuteri*, respectively. Isolated bacteria had no significant effect on the dry matter of SB ($P > 0.05$). The treatments decreased the amount of ash, neutral detergent fiber (NDF) and acidic detergent fiber (ADF) and increased the amount of organic matter (OM) in SB ($P < 0.05$). The results of the gas production test showed that the actual digested OM, microbial mass production, PF, potential and gas production were similar among treatments ($P > 0.05$). The NDF and ADF digestibility were not different between treatments ($P > 0.05$). Therefore, *Enterococcus durans* and *Lactobacillus reuteri*, isolated from RCM and FCM, improved the OM content of SB while decreasing its NDF and ADF concentrations. Therefore, the isolated bacteria have the potential to be used as additives to enhance the digestion of fibrous components in SB, making it more suitable for ruminant feed.

Keywords: Camel milk; lactic acid; sugarcane bagasse; *enterococcus durans; limosilactobacillus reuteri.*

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Introduction

Population growth and a decrease in production of milk make it important to pay attention to alternative protein sources of cow's milk. Due to its non-protein bound amino acids, camel milk is easily digested by microorganisms and when used in the preparation of starter culture or other fermented products, it will achieve more metabolic activity for bacteria. Lactic acid bacteria have been introduced as the predominant microbial flora of camel milk, which can be used in dairy processing technology. This microbial flora may have antitumor activity, reduce serum cholesterol, reduce and treat lactose intolerance and deficiency, stimulate the immune system, and stabilize the microbial flora of the digestive system (Mohammadabadi, 2020). Due to the similarity of camel milk to human milk and its anti-allergic, anti-diabetic, and anti-cancer properties, it is used for therapeutic purposes in many parts of the world (Khalesi et al., 2017; Mohammadabadi, 2020). With proteins similar to insulin, camel milk is a substitute for insulin in type 1 and 2 diabetes and pregnancy, which supplies about 60% of insulin in type 1 diabetic patients (Mohammadabadi, 2020). The predominant microbial flora of raw milk is lactic acid bacteria (Aziz et al., 2009), and they are among the most abundant bacteria associated with humans and are present on mucosal surfaces and the digestive tract (Wood & Warner, 2003). It also plays a role in the industrial and traditional food fermentation process (Axelsson, 2004) and needs an external source of amino acids or peptides that can be provided through casein proteolysis (Savijoki, 2006). Compared to other animals, camel milk is a more suitable environment for the growth of *Lactobacillus acidophilus* (Rahman et al., 2009). The benefits of the *Lactobacillus plantarum* strain isolated from camel milk are much more suitable for the production of low-fat cheese than commercial varieties (Al-Dhaheri et al. 2017). Proteinase enzymes produced by lactic acid bacteria isolated from camel milk have strong proteolytic activity against cow milk casein (Moslehishad et al., 2013).

Lactic acid bacteria include several bacterial genera in the phylum *Firmicutes*. The main ones are *Lactobacillus, Enterococcus, Pediococcus, Streptococcus, Lactococcus, Melissococcus, Onococcus, Leuconestoc, Tetragenococcus, Vajococcus, Visella* (Ercolini et al. 2001; Holzapfel et al. 2001). The most common strain of lactic acid-producing bacteria is *Lactobacillus plantarum*, which is a facultative heterogenous fermenting *Lactobacillus* (Eccleston, 1999). Available microbial additives include *Lactobacillus acidophilus*, *Lactobacillus buchneri,* and *Lactobacillus plantarum*, which can be used due to their good ability to improve fermentation conditions (Kung, 2001). Elgadi et al. (2008) also identified strains of *Lactobacillus* in raw milk as *Lactobacillus acidophilus.* The species identified in the pit in the study (Rahman et al., 2009) are *Lactobacillus casei, Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus heloticus, Leuconostoc lactis, Lactobacillus brevis, Visalia hellenicus, Lactobacillus delbrueckii* and also *Pediococcus species* were also isolated. The presence of *Enterococcus durans* and *Enterococcus faecium* was shown in the isolation of lactic acid bacteria in Shubat (fermented product of camel milk in China and Mongolia) (Serikbayeva et al., 2005). During an experiment, samples of traditional milk, yogurt, and buttermilk were collected from the cities of Kalibar, Harris, and Varzeghan, and as a result of this investigation, *Lactobacillus brevis*, *Lactobacillus plantrum, Lactobacillus dariogenes, Lactobacillus fermentum, Lactobacillus confucius*, *Lactobacillus vasinostercus, Enterococcus durans,* and *Enterococcus mondetti* were isolated (Narimani et al., 2013). Bhardwaj et al. (2012) also reported *Lactobacillus acidophilus* and *Lactobacillus casei* with 17.37 and 24.35% respectively as the most abundant isolates of a type of traditional dairy product in India. Sugarcane is among the most productive tropical crops, benefiting from numerous agronomic advantages, including high yield potential, adaptability to diverse soil types, pest resistance, perennial growth, and advanced support in the form of improved cultivars and optimized cultivation practices (Presto, 1983). Worldwide, the processing of sugarcane for sugar and ethanol production generates over 279 million tons of solid and liquid waste annually, along with various by-products such as straw, bagasse, press mud, wastewater, bagasse ash, vinasse from ethanol distillation, and molasses. Without proper management, these wastes can pose significant risks to the environment and human health (Ungureanu et al., 2022). A majority of the by-products such as sugarcane bagasse (SB) and sugarcane tops can be utilized as roughage for animals, particularly ruminants.

The primary challenges of using sugarcane byproducts for animal feed are their low nutritional value and poor digestibility due to their high lignin content and minimal nitrogen levels (Chaji et al., 2010). Sugarcane bagasse containing about 50% cellulose, 25% hemicellulose and 25% lignin and Sugarcane top contain 28.2 % Hemicellulose, 32.0% Cellulose, 13.6%, Lignin and 6.7% Silica (Murni et al., 2008; Martinez, 2002). Lignin is a key factor, both physically and chemically, that hinders livestock's ability to digest feed ingredients. Chemically, lignin binds to structural carbohydrates, while physically, it acts as a barrier, preventing rumen microbes from breaking down cell walls. However, it can be recycled to produce value-added product such as protein-enriched animal feed by application feed technology. Several researchers indicates that the pre-treatment of high-fiber feeds can enhance both their nutrient content and digestibility (Chen et al. 2010; Ahmed et al. 2013; Samadi et al., 2016).

Pretreatment of rice straw with lactic acid bacteria resulted in increased CP and DM content, and enhanced *in vitro* digestibility (Chen et al., 2022). According to Chen et al. (2017), the fermentation of sugarcane tops with lactic acid bacteria led to a significant increase in DM content.

Based on the investigation of technological properties, camel milk *enterococci* are suitable candidates for processing camel milk and other dairy products (Davati et al., 2013). In the analysis of lactic acid bacteria of raw camel milk in Mongolia, the microbiota of camel milk included 32 orders, 377 genera, and 652 species, the main orders of which were *Proteobacteria, Bacteroides, Deinococcus thermos, Firmicutes*, and *Actinobacteria* (Zhao et al., 2020). The simultaneous use of several types of bacteria in alfalfa silage has increased the number of acid-producing bacteria and improved fermentation in silage (Wang et al., 2020). We hypothesize that lactic acid bacteria isolated from raw and fermented camel milk enhance the chemical composition and nutrient digestibility of SB. The purpose of this research was to investigate the possibility of isolating and identifying lactic acid-producing bacteria from raw and fermented camel milk and to investigate the effect of these bacteria in the processing of SB fibrous matters.

Materials and methods

Isolation of lactic acid bacteria from raw and fermented camel milk

At first, raw camel milk (RCM) was transported to the laboratory in sterile conditions and under refrigeration conditions in the shortest possible time. Around 50 mL of camel milk was utilized in two forms: raw and fermented. To prepare De Man–Rogosa–Sharpe agar (MRS) broth solution, 5.225 g of MRS broth per 1000 mL of distilled water was considered. The obtained solution was placed in an autoclave at a temperature of 121°C for 90 min (Mohammadabadi et al., 2018). For culturing in MRS broth liquid medium for initial enrichment, 500 μL of each sample were inoculated into 30 mL of MRS broth medium (Madrid-Spain) and placed under anaerobic conditions of CO2 and temperature of 37°C for 24 to 72 h. Sub-culturing was carried out in the second MRS medium on MRS agar culture medium, 5.225 g of MRS, 1.5 g of agar, and 100 mL of distilled water were placed in an Erlenmeyer flask, autoclaved for 15 min at 118°C, and after cooling was poured into the pellet for the formation of solid culture medium. After observing the growth of bacteria, they were transferred to the culture medium containing agar, and the transfer to the solid medium was done by the roll tube method on ice. In the next step, serial dilutions were prepared under anaerobic environments, and 10 μL of each dilution was cultured on a plate containing MRS Agar medium for 24 to 48 h at a temperature of 37°C and under anaerobic conditions. 20 μL RCM and fermented camel milk (FCM) used in the agar culture. The colonies of containing the MRS agar culture medium inoculated with RCM and FCM were inoculated on the pellets containing the MRS agar culture medium and then gram staining was done.

Molecular identification

Genome extraction was done manually, and its quality was checked. The general primers were able to amplify the genome in the 16S rRNA gene region, and the primers were synthesized by Dena zist. The sequence of primers is given in Table 1.

Table 1. The sequence of primers.

5-CCGAATTCGACAACAGAGTTTGATCCTGGCTCAG-3	37 bases	Forward primer
5-CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC-3	33 bases	Reverse primer

For the polymerase chain reaction, a stock containing master mix 2X, primer, and water was prepared (Table 2).

Table 2. Components and optimal amounts in the polymerase chain reaction mixture.

Amount used	Final concentration	PCR material
1	pmol 0.4	Primer
1	pmol 0.4	Primer
1	-	Genome
12.5	1x	master mix 2X
9.5	-	Water
25		Final Volume

The temperature program for the amplification of the sequence in the polymerase chain reaction is also shown in the Table 3.

Table 3. Polymerase chain reaction program.

Levels		Temperature (°C)	Time	Number of cycles
First stage		94	5 m	1
Second step	Denaturation	94	1 m	25
	Annealing	55	40 s	
	Synthesis	72	120 s	
Third level		72	10 s	1

Then an agarose gel was prepared and electrophoresis was performed. By observing specific single bands, the PCR product was sent to Gene Fanaveran for sequencing. The sequencing results were sorted using Bio Edit software and analyzed by the BLAST program available on the NCBI. Genomic sequences obtained from sequencing program were recorded in the gene bank. After the sequence was confirmed by the gene bank, their accession number was received.

Processing of fibrous materials with isolated bacteria

To process the culture medium containing bacteria isolated from RCM and FCM on SB, 25 cc of the culture medium containing bacteria isolated from RCM and FCM mixed with 500 cc of distilled water; then 300 g of SB was mixed with a culture medium solution containing bacteria isolated from RCM and FCM. Then 100 g of all the samples were put in dark plastic and pressed well to remove the excess air, and were kept in darkness for 4 days. The prepared solution was poured into 300 g of the control sample and fully compressed to remove excess air (Shakarami et al., 2019).

The chemical properties of experimental samples were determined as neutral detergent fiber (NDF) according to the method of Van Soest et al. (1991) and acidic detergent fiber (ADF), dry matter and ash based on Association of Official Analytical Chemists, (1990). To determine the fermentation and digestion of processed samples, gas production test was used (Menke and Steingass, 1988). The two-step digestion method was used to determine digestibility (Tilley & Terry, 1963).

Statistical analyses

The data obtained were analyzed with the statistical software SAS version 9.4 using the generalized linear model procedure according to the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$; where Y_{ij} is the observation, μ represents the mean, T_i shows the effect of isolated bacteria on substrates and e_{ij} illustrates the standard error of term. Means were compared by Duncan's multiple range test at the 5% probability level.

Results

Isolated bacteria

The bacteria isolated from RCM was 99.33% similar to *Enterococcus durans* and gene bank number OP265886 (Figure 1). The bacteria isolated from FCM was 99.42% similar to *Lactobacillus limosi* with gene bank number OP265887 (Figure 2).

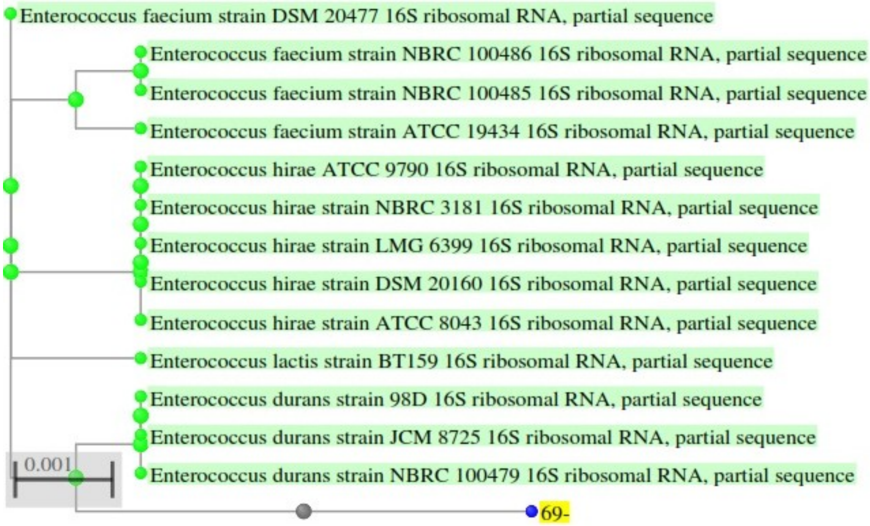

Figure 1. The lactic acid bacteria isolated from raw camel milk (RCM).

The effect of isolated bacteria on the chemical composition of SB

The chemical composition of SB pre-treated with lactic acid bacteria isolated from RCM and FCM is presented in Table 4. There was no significant effect on the dry matter of SB processed with bacteria isolated from RCM and FCM ($P < 0.05$). The processing of SB with isolated bacteria significantly decreased ash and increased organic matter content ($P < 0.05$). The amount of neutral detergent fiber in treatment with bacteria isolated from RCM and FCM was 91.77 and 23.91 respectively and the amount of acidic detergent fiber decreased significantly by isolated bacteria ($P < 0.05$).

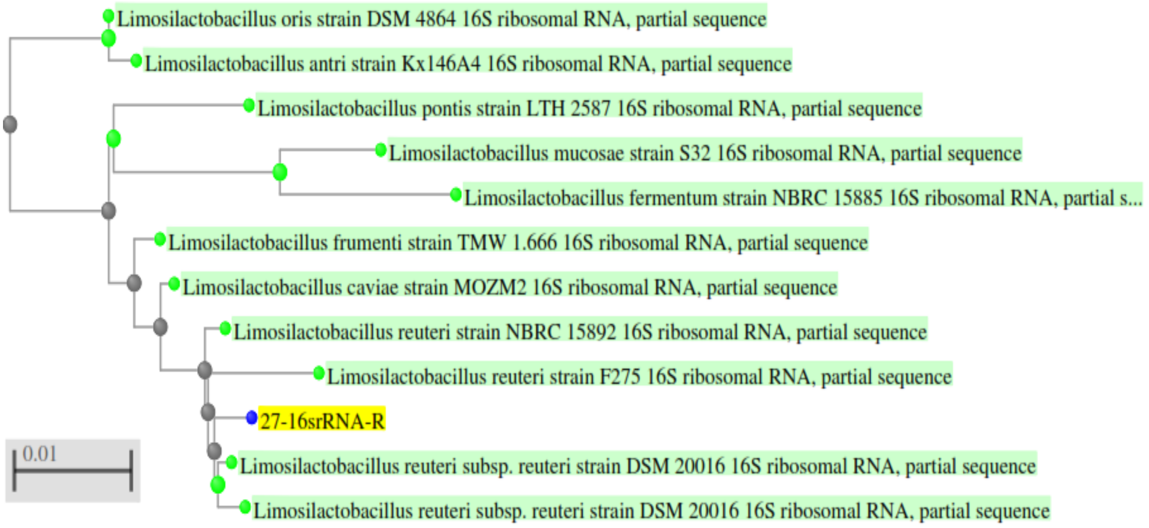

Figure 2. The lactic acid bacteria isolated from fermented camel milk (FCM).

Table 4. The effect of lactic acid bacteria isolated from raw (RCM) and fermented camel milk (FCM) on the chemical composition of sugarcane bagasse.

Chemical composition	Treatment*			SEM	*P*-value
	Control	SB-FCM	SB-RCM		
Dry matter	75.81	75.68	75.46	2.29	0.14
Organic matter	93.26^{b}	94.18^{a}	94.42^{a}	0.2	0.0041
NDF	44.25^{a}	41.32^{b}	41.77^{b}	0.62	0.0128
ADF	30.97a	26.53b	24.55^{b}	1.12	0.0048
Ash	6.73^{a}	5.82^{b}	5.57^{b}	0.2	0.00041

^{a,b,c}In each rows, values with different letters are significantly different (P < 0.05). *Control: sugarcane bagasse with no processing, SB-FCM: sugarcane bagasse processed with lactic acid bacteria isolated form fermented camel milk and SB-RCM: sugarcane bagasse processed with lactic acid bacteria isolated form raw camel milk. NDF, natural detergent fiber, ADF, acid detergent fiber.

The effect of isolated bacteria on gas production and digestibility of SB

The digested OM, microbial biomass, partitioning factor, as well as the gas production potential and rate of SB, were not significantly affected by bacteria isolated from RCM and FCM ($P > 0.05$; Table 5). The digestibility of dry matter, NDF and ADF of SB was not different between treatments ($P < 0.05$).

Table 5. The effect of lactic acid bacteria isolated from raw (RCM) and fermented camel milk (FCM) on gas production and digestibility of sugarcane bagasse.

Gas production parameters	Treatment*			SEM	*P*-value
	Control	SB-FCM	SB-RCM		
Actual digested organic matter (mg)	30.19	31.38	30.41	1.04	0.49
Microbial biomass	16.08	16.51	16.86	1.67	0.49
Partitioning factor (mg mL^{1})	4.96	5.44	5.01	0.43	0.69
Gas production potential (mL)	77.18	81.30	80.57	2.9	0.55
Gas production rate (mL)	0.021	0.025	0.025	0.003	0.72
Dry matter	51.76	51.93	51.98	1.10	0.98
NDF	33.08	34.52	35.04	1.41	0.89
ADF	31.10	32.67	32.16	0.76	0.39

*Control: sugarcane bagasse with no processing, SB-FCM: sugarcane bagasse processed with lactic acid bacteria isolated form fermented camel milk and SB-RCM: sugarcane bagasse processed with lactic acid bacteria isolated form raw camel milk. NDF, natural detergent fiber, ADF, acid detergent fiber.

Discussion

Isolated bacteria

Enterococci are Gram-positive and catalase-negative bacteria, anaerobic, spore less, capsule-less cocci (Gomes et al., 2008); De Roos et al., 1998) that inhabit the digestive tract of humans, animals and plants (Chen et al., 2003). Due to high heat resistance, they form part of the microflora of raw and pasteurized milk (Gelsomino et al., 2001) and produce lactate from glucose fermentation.

Further, *Enterococcus durans* and *Enterococcus faecium* isolated from camel milk have good autolysis power and the difference in the intensity of autolysis among the strains comes from their wide variety. The diversity of strains to release intracellular enzymes during cheese ripening is considered a desirable feature in the industry (Wilkinson et al., 1994). The lactobacilli in raw camel milk of Iranian one-humped included *Enterococcus durans, Enterococcus mondeti, Enterococcus faecium, Enterococcus doris, Enterococcus avium and Enterococcus faecalis.* These strains have a high-ability to produce acid and lipolytic activity. *Enterococcus faecalis* showed the highest lipolytic activity compared to other isolates, and *Enterococcus faecium* and *Enterococcus faecalis* showed the highest amount of autolysis among other samples (Davati et al., 2013).

The lactic acid bacteria of RCM in Mongolia included *Streptococcus thermophilus*, *Lactobacillus lactis, Lactobacillus heloticus* and *Leuconostoc mesenterioides*, and a total of 72 strains of lactic acid bacteria including *Lactobacillus paracasei, Enterococcus durans, Lactococcus lactis, Enterococcus italicus and Enterococcus faecium* were isolated and identified (Zhao et al., 2020).

Wang et al. (2016) isolated 202 lactic acid isolates from 66 yogurt samples in Mongolia, which were divided into 4 genera: Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, and 21 species and subspecies. The variety of lactic acid bacteria found in dairy products made from milk can be a good source of probiotic bacteria. In a study on the taxonomy and physiology of probiotic lactic acid bacteria, after gene amplification and sequencing of PCR product, 2 species of *Enterococcus faecium* were identified. Probiotic enterococci have been isolated from various dairy products and processed products such as sausages (Klein et al., 1998).

The dominant species of lactic acid bacteria isolated from traditional cheese of Semnan (Khiki) included *Lactobacillus plantarum, Lactobacillus delbrocci*, *Lactobacillus heloticus, Lactobacillus casei, Lactobacillus rhamnosus*, and the dominant species of enterococcus were *Enterococcus faecium* (74.47%) and *Enterococcus durans* (25.53%).

Lactobacillus limosi is a lactic acid bacterium that exists as a probiotic in humans, pigs, goats and mice, in the digestive tract, breast milk, and the mouth, which prevents bone loss and improves the immune system (Luo et al., 2021). *Lactobacillus roteri*, which is one of the normal flora of the digestive system of animals, easily forms a colony in the intestine and inhibits the growth of pathogenic bacteria. On the other hand, it is used as probiotic bacteria in yogurt and cheese, dry products or frozen foods such as milk powder, ice cream, cereals, beans and chocolate (Huang et al., 2021). This bacterium is one of the few natural Lactobacillus bacteria found in human intestines (Casas and Dobrogosz, 2000).

Mohammadi et al. (2018) reported that 16 strains of *Lactobacillus roteri* and 2 strains of *Lactobacillus gasri* were isolated from breast milk. Investigating of the diversity of lactic acid bacteria isolated from Zaboli yellow curd (Sistani) by using 16S rDNA sequencing; *Lactobacillus plantarum*, *Lactobacillus helviticus, Lactobacillus brevis, Lactobacillus delbruecki subsp. mesenteroides*, *Leuconostoc citreum,* and *Pediococcus pentozaceus* were identified.

About 62 x 10^6 cfu m^1 lactobacilli in the traditional yogurts in the villages of East Azarbaijan province was determined. In one study, 9 lactic acid bacteria with probiotic properties were isolated from 32 traditional yogurts of Varamin city, including 7 isolates of lactic acid bacteria from cow yogurt and 2 isolates from sheep yogurt. The sequencing results of three bacterial isolates showed the presence of one *Lactobacillus casei* and two *Enterococcus faecium* bacteria. In the current study, most of the isolated lactic acid isolates had probiotic potential that bile salt tolerance and acid tolerance tests were positive (Yaghoubi, 2019).

In one research, samples of raw milk were prepared from 14 points of Alborz highlands were contained 6 genera, 9 species and 2 new species. The findings include *Lactobacillus plantrum*, *Lactobacillus rhamnosus, Lactococcus lactis*, *Enterococcus faecalis, Streptococcus vestibularis, Lactobacillus brevis, Leuconostoc argentinum, Streptococcus infantarius, Pediococcus pentosacea* and two bacteria from the Enterococcus and Pediococcus genera were identified based on 16s rDNA analysis. The results show that there is a valuable variety of raw milk LAB of Central Alborz (Forghani et al., 2010).

The effect of isolated bacteria on chemical composition of SB

The results of this experiment showed that there was no significant effect on the dry matter of SB processed with bacteria isolated from RCM and FCM. In the report of Kazemi et al. (2012), the amount of dry matter of alfalfa silage treated with ecosyle *Lactobacillus plantarum* was significantly different compared to the treatments processed with Lactisyl Maize and ecosyle that reduced dry matter (Kung, 1997). The processing of corn silage with *Lactobacillus plantrarum* bacteria did not have a significant difference in the amount of dry matter (Abdul Rahman et al., 2017). The using of heterogeneous lactic acid producing bacteria

(*Lactobacillus buchneri*) for alfalfa and orange pomace was not affected dry matter. Also, the use of a bacterial additive caused a decrease in the amount of soluble carbohydrates compared to the alfalfa and orange pomace without additives; also, the bacterial additive caused a significant decrease in pH compared to the control treatment; this decreasing trend in pH increased numerically with the increase in the used additive. In general, the reason for the decrease in pH compared to the control treatment is related to the high level of soluble carbohydrates and the availability of more nutrients for the population of lactic acid producing bacteria (Besharatı et al., 2020). In the research of Mohammadzadeh (2012) regarding the multi-strain microbial additive on the fermentation of corn silage and the performance of Holstein dairy cows, there was no significant difference between the dry matter content of the control silage and the additive-treated silage. Inoculation of *Lactobacillus plantrum* bacterium to corn silage reduced the ash concentration compared to the control treatment (Abdul Rahman et al., 2017). The addition of *Lactobacillus Buchneri* and *Pediococcus lactici* in hay ensiling on the performance of fattening male lambs did not show an effect on the ash (Rezaian et al., 2018). Kamarloy and Teimouri (2008) reported bacterial additives on the corn silage with *Lactobacillus plantrum* and *Propionibacterium* increased ash of barley silage. In the present trial, OM showed a significant increase in treated groups, particularly in SB-RCM (94.42%) and SB-FCM (94.18%) compared to the control (93.26%). This increase may be attributed to the reduction in ash and the enhancement of organic constituents during microbial processing. There was no significant difference between the treatments in organic matter of corn silage processed with *Enterococcus faecium*, *Lactobacillus plantarum, Pediococcus pentosaceus, Lactobacillus casei* and *Lactobacillus buchneri*. In alfalfa silages treated with *Lactobacillus acidophilus*, *Saccharomyces servicia and Aspergillus aureus*, organic matter and ash were not affected by microbial additives (Delavar et al., 2012). The amount of ash and organic matter of tomato pulp ensiled with beet pulp and *Lactobacillus plantarum* bacteria on the performance of Mughani fattening lambs did not change (Yanegh et al., 2019). There was no significant difference in organic matter between alfalfa inoculated with Lactisel Maize, Lacil and the control treatment (Kazemi et al., 2012).

Mohammadzadeh et al. (2013) reported the amount of NDF was the highest in lactisil maize containing *Lactobacillus plantrum, Lactobacillus casei, Pediococcus pentosaceus, Enterococcus faecium and Lactobacillus buchandri* which is probably due to consuming more soluble sugars and the increase the fermentation rate. The effect of additives reduced the amount of ADF. The addition of heterogeneous lactic acid producing bacteria (*Lactobacillus buchneri*) on alfalfa did not change NDF and decreased ADF (Karimi et al., 2016). The amount of NDF of silage alfalfa treated with of ecosyl and lactisil containing *Enterococcus faecium*, *Lactobacillus plantrum*, *Pediococcus pentosaceus*, *Lactobacillus casei* and *Lactobacillus bucheneri* was not different (Kazemi et al., 2012). Also, in the report of Kamarloy and Teimouri (2008), NDF was not different between control silage and silage inoculated with *Lactobacillus plantrum* and Propionibacterium bacteria on beef cattle. Alfalfa silages treated with *Lactobacillus acidophilus*, *Saccharomyces servicii* and *Aspergillus aureus* caused a significant decrease in NDF and ADF (Delavar et al., 2012). Tian et al. (2024) reported that parameters of gas production and, digestibility of triticale silage fodder with ecozyle was a significant difference in digestibility of organic matter (Table 4). Overall, the data suggest that lactic acid bacteria, especially from RCM, effectively alter the chemical composition of sugarcane bagasse, primarily by reducing fiber and ash content while increasing OM. These changes could improve the digestibility and nutritional quality of SB for use in animal feed.

The effect of isolated bacteria on gas production and digestibility of SB

Differences in gas production typically reflect variations in the chemical makeup of feed components and are widely utilized to assess their nutritional quality. According to Getachew et al. (1998), gas production also serves as an indicator of short-chain fatty acid synthesis and microbial protein formation. During fermentation, microbial degradation of carbohydrates and proteins generates metabolites such as acetate, butyrate, and ammonia, all of which contribute to overall gas output. The results show that none of the measured variables differed significantly among treatments. Although not statistically significant, some numerical trends were observed. The actual digested OM was slightly higher in the SB-FCM group (31.38 mg) compared to the control (30.19 mg) and SB-RCM (30.41 mg), indicating a marginal improvement in OM degradability with fermentation, particularly using bacteria from FCM. Tian et al. (2024), no significant difference was observed in the amount of gas produced and the gas production parameters of triticale silages with bacterial additives. Using 14 strains of *Lactobacillus plantarum* resulted in increased gas production compared to the control (Astuti et al., 2018). Lactic acid producing and consuming bacteria and *Saccharomyces cerevisiae* increased significantly the amount of gas produced at all times. The use of microbial food additives improved digestibility of organic matter and metabolizable energy (Direkvandi et al., 2018). The lactic acid producing bacteria and urea on dry matter digestibility of whole barley fodder was not effective (Roghani Haghighi Fard et al., 2002). The lactic acid-producing and utilizing bacteria and *Saccharomyces servicia* in a high concentrate diet in Arabian sheep, was not affected dry matter digestibility. Also, the use of *Lactobacillus plantarum* as a bacterial additive increased the *in vitro* dry matter digestibility of the silage grasses, due to the reduction of the cell wall and neutral detergent fibers by bacterial inoculation (Jin et al., 2024) (Table 5). Overall, Although the application of lactic acid bacteria from camel milk did not result in statistically significant improvements in gas production or digestibility parameters, the numerical trends observed suggest a potential for enhancing OM degradation and promoting microbial biomass development.

Conclusion

The bacteria isolated from RCM had 33.99% similarity to *Enterococcus durans* with gene bank number OP265886 and the bacteria isolated from FCM has 99.42% similarity to *Limosilactobacillus reuteri* with the gene bank number OP265887. Therefore, *Enterococcus durans* and *Lactobacillus reuteri* isolated from RCM and FCM enhance OM content while reducing NDF and ADF content of SB. Future *in vivo* studies are warranted to evaluate the effects of these isolated bacterial strains on the performance and nutrient digestibility of ruminants.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Abdul Rahman, N., Abd Halim, M. R., Mahawi, N., Hasnudin, H., Al-Obaidi, J. R., & Abdullah, N. (2017). Determination of the use of *Lactobacillus plantarum* and *Propionibacterium freudenreichii* application on fermentation profile and chemical composition of corn silage. *Biomed Research International*, *2017*, Artigo 2038062. https://doi.org/10.1155/2017/2038062

Ahmed, M. H., Babiker, S. A., Elseed, A. E. M. A., & Mohammed, A. M. (2013). Effect of urea-treatment on nutritive value of sugarcane bagasse. *ARPN Journal of Science and Technology*, *3*(8), 834–838.

Al-Dhaheri, A. S., Al-Hemeiri, R., Kizhakkayil, J., Al-Nabulsi, A., Abushelaibi, A., Shah, N. P., & Ayyash, M. (2017). Health-promoting benefits of low-fat Akawi cheese made by exopolysaccharide-producing probiotic *Lactobacillus plantarum* isolated from camel milk. *Journal of Dairy Science*, *100*(10), 7771–7779. https://doi.org/10.3168/jds.2017-12761

Association of Official Analytical Chemists [AOAC]. (1990). *Official methods of analysis* (15th ed.). AOAC.

Astuti, W. D., Wiryawan, K. G., Wina, E., Widyastuti, Y., Suharti, S., & Ridwan, R. (2018). Effects of selected *Lactobacillus plantarum* as probiotic on in vitro ruminal fermentation and microbial population. *Pakistan Journal of Nutrition*, *17*(3), 131–139. https://doi.org/10.3923/pjn.2018.131.139

Axelsson, L. (2004). Lactic acid bacteria: Classification and physiology. Em S. Salminen, A. von Wright, & A. Ouwehand (Orgs.), *Lactic acid bacteria: Microbiological and functional aspects* (3rd ed., pp. 1–66). Marcel Dekker.

Aziz, T., Khan, H., Bakhtair, S. M., & Naurin, M. (2009). Incidence and relative abundance of lactic acid bacteria in raw milk of buffalo, cow and sheep. *Journal of Animal and Plant Sciences*, *19*(4), 168–173.

Besharati, M., Karimi, M., Taghizadeh, A., Nemati, Z., & Kaygısız, A. (2020). Improve quality of alfalfa silage ensiled with orange pulp and bacterial additive. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *23*(6), 1669–1677. https://doi.org/10.18016/ksutarimdoga.vi.673623

Bhardwaj, A., Puniya, M., Sangu, K. P. S., Kumar, S., & Dhewa, T. (2012). Isolation and biochemical characterization of *Lactobacillus* species isolated from Dahi. *Research & Reviews: A Journal of Dairy Science and Technology*, *1*(2), 18–31.

Casas, I. A., & Dobrogosz, W. J. (2000). Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microbial Ecology in Health and Disease*, *12*(4), 247–285. https://doi.org/10.1080/08910600050216246

Chaji, M., Naserian, A. A., Valizadeh, R., Mohammadabadi, T., & Mirzadeh, K. (2010). Potential use of high-temperature and low-temperature steam treatment, sodium hydroxide and an enzyme mixture for improving the nutritional value of sugarcane pith. *South African Journal of Animal Science*, *40*(1), 22–32.

Chen, H., & Hoover, D. G. (2003). Bacteriocins and their food applications. *Comprehensive Reviews in Food Science and Food Safety*, *2*(3), 82–100. https://doi.org/10.1111/j.1541-4337.2003.tb00016.x

Chen, X., Lawoko, M., & van Heiningen, A. (2010). Kinetics and mechanism of autohydrolysis of hardwoods. *Bioresource Technology*, *101*(20), 7812–7819. https://doi.org/10.1016/j.biortech.2010.05.006

Chen, X., Li, W., Gao, C., Zhang, X., Weng, B., & Cai, Y. (2017). Silage preparation and fermentation quality of kudzu, sugarcane top and their mixture treated with lactic acid bacteria, molasses and cellulase. *Animal Science Journal*, *88*(11), 1715–1721. https://doi.org/10.1111/asj.12844

Chen, X., Ma, Y., Khan, M. Z., Xiao, J., Alugongo, G. M., Li, S., Wang, J., & Cao, Z. (2022). A combination of lactic acid bacteria and molasses improves fermentation quality, chemical composition, physicochemical structure, in vitro degradability and rumen microbiota colonization of rice straw. *Frontiers in Veterinary Science*, *9*, Artigo 900764. https://doi.org/10.3389/fvets.2022.900764

Davati, N., Tabatabai Yazdi, F., & Zibaee, S. (2013). Isolation and identification of lactobacilli from Iranian one-humped camel raw milk and investigation of their technological properties. *Journal of Veterinary Microbiology*, *9*(2), 79–90.

De Roos, N. M., & Katan, M. B. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: A review of papers published between 1988 and 1998. *The American Journal of Clinical Nutrition*, *71*(2), 405–411. https://doi.org/10.1093/ajcn/71.2.405

Delavar, M., Tahmasabi, A., & Valizadeh, R. (2012). The effect of harvesting time, duration of ensiling and applications of microbial additives on the chemical components of alfalfa silage. *Journal of Animal Science Research (Pajouhesh-va-Sazandegi)*, *4*(2), 144–154.

Direkvandi, E., Mohammadabadi, T., & Zidan, M. A. F. (2018). Investigating the effect of lactic acid producing and consuming bacteria and *Saccharomyces servicii* on parameters of gas production, ruminal fermentation and digestibility of nutrients in a high-concentrate diet by in vitro method in Arabian sheep. *Journal of Research on Ruminants*, *7*(4), 111–129.

Eccleston, C. H. (1999). *The NEPA planning process: A comprehensive guide with emphasis on efficiency*. John Wiley & Sons.

Elgadi, Z. A. M., Gadir, W. S. A., & Dirar, H. A. (2008). Isolation and identification of lactic acid bacteria and yeast from raw milk in Khartoum State (Sudan). *Research Journal of Microbiology*, *3*(3), 163–168.

Ercolini, D., Moschetti, G., Blaiotta, G., & Coppola, S. (2001). Behavior of variable V3 region from 16S rDNA of lactic acid bacteria in denaturing gradient gel electrophoresis. *Current Microbiology*, *42*(3), 199–202. https://doi.org/10.1007/s002840010204

Forghani, F., Nazemi, A., Sharifi, Sh., & Eskandari, M. (2010). Isolation and molecular identification of lactic acid bacteria in raw milk from the Central Alborz highlands using 16S rDNA PCR Sequencing and High Resolution Melting Real Time PCR. *Scientific and Research Journal of Microbial Biotechnology*, *Islamic Azad University*, 2(5), 21-28.

Gelsomino, R., Vancanneyt, M., Condon, S., Swings, J., & Cogan, T. M. (2001). Enterococcal diversity in the environment of an Irish Cheddar-type cheesemaking factory. *International Journal of Food Microbiology*, *71*(2-3), 177–188. https://doi.org/10.1016/s0168-1605(01)00620-1

Getachew, G., Blümmel, M., Makkar, H. P. S., & Becker, K. (1998). In vitro gas measuring techniques for assessment of nutritional quality of feeds: A review. *Animal Feed Science and Technology*, *72*(3-4), 261–281. https://doi.org/10.1016/S0377-8401(97)00189-1

Gomes, B. C., Esteves, C. T., Palazzo, I. C., Darini, A. L. C., Felis, G. E., Sechi, L. A., Franco, B. D., & De Martinis, E. C. (2008). Prevalence and characterization of *Enterococcus* spp. isolated from Brazilian foods. *Food Microbiology*, *25*(5), 668–675. https://doi.org/10.1016/j.fm.2008.03.008

Holzapfel, W. H., Haberer, P., Geisen, R., Björkroth, J., & Schillinger, U. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. *The American Journal of Clinical Nutrition*, *73*(2), 365S–373S. https://doi.org/10.1093/ajcn/73.2.365s

Huang, X., Gänzle, M., Zhang, H., Zhao, M., Fang, Y., & Nishinari, K. (2021). Microencapsulation of probiotic lactobacilli with shellac as moisture barrier and to allow controlled release. *Journal of the Science of Food and Agriculture*, *101*(2), 726–734. https://doi.org/10.1002/jsfa.10685

Jin, Y., Wang, P., Li, F., Yu, M., Du, J., Zhao, T., Yi, Q., Tang, H., & Yuan, B. (2024). The effects of *Lactobacillus plantarum* and *Lactobacillus buchneri* on the fermentation quality, in vitro digestibility, and aerobic stability of *Silphium perfoliatum* L. silage. *Animals*, *14*(15), Artigo 2279. https://doi.org/10.3390/ani14152279

Kamarloiy, M., & Yansari, A. T. (2008). Effect of microbial inoculants on the nutritive value of corn silage for beef cattle. *Pakistan Journal of Biological Sciences*, *11*(8), 1137–1141. https://doi.org/10.3923/pjbs.2008.1137.1141

Karimi, M., Besharati, M., & Taghizadeh, A. (2016). Effect of heterogeneous lactic acid producing bacterial additive on the chemical composition and fermentation properties of pulverized alfalfa with orange pomace. *Animal Production Research*, *5*(1), 27–36.

Kazemi, F., Dehghan-Banadaki, M., Zali, A., & Rezayazdi, K. (2012). Effect of bacterial additive on nutritional value and rumen degradability of alfalfa silage. *Iranian Journal of Animal Science*, *43*(3), 327–335.

Khalesi, M., Salami, M., Moslehishad, M., Winterburn, J., & Moosavi-Movahedi, A. A. (2017). Biomolecular content of camel milk: A traditional superfood towards future healthcare industry. *Trends in Food Science & Technology*, *62*, 49–58. https://doi.org/10.1016/j.tifs.2017.02.004

Klein, G., Pack, A., Bonaparte, C., & Reuter, G. (1998). Taxonomy and physiology of probiotic lactic acid bacteria. *International Journal of Food Microbiology*, *41*(2), 103–125. https://doi.org/10.1016/s0168-1605(98)00049-x

Kung, L., Jr., & Muck, R. E. (1997). Animal response to silage additives. *Proceedings of the Silage: Field to Feedbunk North American Conference* (pp. 200–210). NRAES.

Kung, L., Jr., & Ranjit, N. K. (2001). The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *Journal of Dairy Science*, *84*(5), 1149–1155. https://doi.org/10.3168/jds.s0022-0302(01)74575-4

Luo, H., Li, P., Wang, H., Roos, S., Ji, B., & Nielsen, J. (2021). Genome-scale insights into the metabolic versatility of *Limosilactobacillus reuteri*. *BMC Biotechnology*, *21*(1), Artigo 46. https://doi.org/10.1186/s12896-021-00702-w

Menke, K. H., & Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Development*, *28*, 7–55.

Mohammadabadi, T. (2020). Camel milk as an amazing remedy for health complications: A review article. *Basrah Journal of Agricultural Sciences*, *33*(2), 113–126. https://doi.org/10.37077/25200860.2020.33.2.10

Mohammadabadi, T., Bakhtiari, M. A., & Alimirzaei, P. (2018). Isolation and identification of lactate-producing and utilizing bacteria from the rumen of Najdi goats. *Indian Journal of Small Ruminants*, *24*(2), 276–280. https://doi.org/10.5958/0973-9718.2018.00056.9

Mohammadi, F., Eshaghi, M., Razavi, S., Darban Sarokhalil, D., Talebi, M., & Pourshafie, M. R. (2018). Characterization of bacteriocin production in *Lactobacillus* spp. isolated from mother's milk. *Microbial Pathogenesis, 118,* 242–246. https://doi.org/10.1016/j.micpath.2018.03.020

Mohammadzadeh, H. (2012). Effects of lactic acid bacteria inoculant on fermentation, nutritive value and aerobic stability of corn silage and Holstein dairy cattle performances. *Journal of Animal Science Research*, *22*(1), 35–44.

Mohammadzadeh, H., Khorvash, M., Ghorbani, G. R., & Yang, W. Z. (2013). Frosted corn silage with or without bacterial inoculants in dairy cattle ration. *Livestock Science*, *145*(1-3), 153–159. https://doi.org/10.1016/j.livsci.2012.01.011

Moslehishad, M., Mirdamadi, S., Ehsani, M. R., Ezzatpanah, H., & Moosavi-Movahedi, A. A. (2013). The proteolytic activity of selected lactic acid bacteria in fermenting cow's and camel's milk and the resultant sensory characteristics of the products. *International Journal of Dairy Technology*, *66*(2), 279–285. https://doi.org/10.1111/1471-0307.12017

Murni, R., Suparjo, Akmal, & Ginting, B. L. (2008). *Teknologi Pemanfaatan Limbah untuk Pakan*. Laboratorium Makanan Ternak, Universitas Jambi.

Narimani, T., Tarinegad, A., & Hejazi, M.A. (2013). Isolation and identification of lactic acid bacteria from traditional dairy products of Kieibar, Herus and Varzaghan. *Journal of Food Science*, 3(3), 23-37.

Perez, J., Muñoz-Dorado, J., de la Rubia, T., & Martinez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. *International Microbiology*, *5*(2), 53–63. https://doi.org/10.1007/s10123-002-0062-3

Preston, T. R. (1983). *Sugarcane by-products as livestock feed*. http://www.fao.org/3/x5514e/x5514e05.htm

Rahman, N., Chen, X., Fu, M., & Ding, M. (2009). Characterization of the dominant microflora in naturally fermented camel milk shubat. *World Journal of Microbiology and Biotechnology*, *25*(11), 1941–1946. https://doi.org/10.1007/s11274-009-0092-5

Rezaian, M., Nafarieh, N., Mahmoudzadeh, H., & Honarzad, J. (2018). The effect of adding *Lactobacillus buchneri and Pediococcic acidosus lacticus* on alfalfa silage fermentation parameters and the effect of its consumption on the performance of fattening male lambs of the Shal breed. *Journal of Applied Microbiology in Food Industries*, 4(4),70-81.

Roghani Haghighifard, E. (2002). The effect of lactic acid and urea producing bacteria on the chemical composition and nutritional value of whole barley forage. *Agricultural Sciences and Technologies and Natural Resources*, *6*(2), 115-124.

Samadi, S., Siti, W., Yunasri, U., Denny, R., & Zidny, A. F. (2016). Improving sugarcane bagasse as animal feed by ammoniation and followed by fermentation with *Trichoderma harzianum* (*In Vitro* Study). *Indonesian Journal of Animal Production*, 18(1),14-21.

Savijoki, K., Ingmer, H., & Varmanen, P. (2006). Proteolytic systems of lactic acid bacteria. *Applied Microbiology and Biotechnology*, *71*(4), 394–406. https://doi.org/10.1007/s00253-006-0427-1

Serikbayeva, A., Konuspayeva, G., Faye, B., Loiseau, G., & Narmuratova, M. (2005). Probiotic properties of a sour-milk product: Shubat from the camel milk. Em B. Faye & P. Esenov (Orgs.), *Desertification combat and food safety: The added value of camel producers* (pp. 187–191). IOS Press.

Shakarami, M. H., Mohammadabadi, T., Motamedi, H., Sari, M., & Teimouri Yansari, A. (2019). Isolation and identification of cellulolytic bacteria from gastrointestinal tract of Arabian horse and investigation of their effect on the nutritional value of wheat straw. *Journal of Applied Microbiology*, *127*(2), 344–353. https://doi.org/10.1111/jam.14304

Tian, J., Tian, R., Wu, J., Huang, L., & Zhang, J. (2024). Gas production characteristics of oats and triticale silages and techniques for reducing gas emissions. *Journal of Integrative Agriculture*, *23*(3), 963–975. https://doi.org/10.1016/j.jia.2023.05.031

Tilley, J. M. A., & Terry, D. R. (1963). A two-stage technique for the in vitro digestion of forage crops. *Grass and Forage Science*, *18*(2), 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Ungureanu, N., Vlăduţ, V., & Biriş, S. S. (2022). Sustainable valorization of waste and by-products from sugarcane processing. *Sustainability*, *14*(17), Artigo 11089. https://doi.org/10.3390/su141711089

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Wang, M., Franco, M., Cai, Y., & Yu, Z. (2020). Dynamics of fermentation profile and bacterial community of silage prepared with alfalfa, whole-plant corn and their mixture. *Animal Feed Science and Technology*, *270*, Artigo 114702. https://doi.org/10.1016/j.anifeedsci.2020.114702

Wang, Y., Zhou, J., Xia, X., Zhao, Y., & Shao, W. (2016). Probiotic potential of *Lactobacillus paracasei* FM-LP-4 isolated from Xinjiang camel milk yoghurt. *International Dairy Journal*, *62*, 28–34. https://doi.org/10.1016/j.idairyj.2016.07.001

Wilkinson, M. G., Guinee, T. P., O'Callaghan, D. M., & Fox, P. F. (1994). Autolysis and proteolysis in different strains of starter bacteria during Cheddar cheese ripening. *Journal of Dairy Research*, *61*(2), 249–262. https://doi.org/10.1017/s0022029900028260

Wood, B. J. B., & Warner, P. J. (2003). *Genetics of lactic acid bacteria* (Vol. 3). Springer. https://doi.org/10.1007/978-1-4615-7090-5

Yaghoubi, F., Honarmand Jahromi, S., & Baghbani Arani, F. (2019). Isolation and identification of lactic acid bacteria with probiotic properties from traditional yogurts of Varamin city. *Iranian Journal of Biological Science*,14(1), 9-18.

Yanegh, A., Ghanbari, F., BayatKouhsar, J., & Moslemipur, F. (2019). Chemical composition and gas production parameters of tomato pomace ensiled with sugar beet pulp and bacterial inoculation and its effect on performance of Moghani fattening lambs. *Animal Science Journal (Pajouhesh-va-Sazandegi)*, *32*(122), 247–262. https://doi.org/10.22092/asj.2018.121543.1678

Zhao, J., Fan, H., Kwok, L. Y., Guo, F., Ji, R., Ya, M., & Chen, Y. (2020). Analyses of physicochemical properties, bacterial microbiota, and lactic acid bacteria of fresh camel milk collected in Inner Mongolia. *Journal of Dairy Science*, *103*(1), 106–116. https://doi.org/10.3168/jds.2019-17070

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