



Short communication

Characteristics of Korean-Saenen goat milk caseins and somatic cell counts in comparison with Holstein cow milk counterparts

J.-S. Ham^a, S.-G. Lee^a, S.-G. Jeong^a, M.-H. Oh^a, D.-H. Kim^a, Y.W. Park^{b,*}

^a Animal Food Processing Division, National Institute of Animal, Science-RDA, Suwon, Gyeonggi 441-706, Republic of Korea

^b Georgia Small Ruminant Research and Extension Center, Agriculture Research Station, Fort Valley State University, Fort Valley, GA 31030-4313, USA

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ABSTRACT

Characteristics of α - and β -casein fractions in the milk of Korean-Saenen goats were compared with those of Holstein cow milk using capillary electrophoresis (CE) analysis. The α_{S1} -CN content of the Saenen goat milk samples varied from 2.4% to 9.3% of total proteins. Total α -CN content of the goat milk varied from 10.1% to 17.0%. Total β -CN content containing β_1 -CN and the β_2 -CN varied from 49.6% to 61.0% of total proteins. Average α -CN to β -CN ratio of the Saenen goat milk from different farms was 0.24 ± 0.04 , ranging from 0.17 to 0.33. The α -CN (α_{S1} -CN + α_{S0} -CN) to β -CN (β_{A1} -CN + β_{A2} -CN) ratio of Holstein cow milk was 0.81, which was much higher than that of Korean-Saenen goat milk. The goat milk samples having more than 1.5 million cells/ml somatic cell counts (SCC) contained higher α -CNs ($P < 0.01$) and lower β -CNs ($P < 0.05$) contents than milks with < 1.5 million SCC. This resulted in a higher α -CN to β -CN ratio ($P < 0.01$) in the milk with > 1.5 million SCC.

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1. Introduction

Dairy goats were introduced to Korea in the early 1960s after the Korean War, mainly Saenen breed imported from Australia and New Zealand. While the dairy goat industry is young and very small, annual importation of goat milk powder to South Korea has been more than \$50 million, stemming from the preference of goat formula milk over cow milk counterparts by mothers as well as general consumers primarily due to hypoallergenic nature of goat milk.

Goat milk has been recommended as a substitute of cow milk for infants and patients who suffer from cow milk allergy (CMA), since cow milk proteins cannot be tolerated by the CMA patients (Park, 1994; Park and Haenlein, 2006). The low level of α_{S1} -casein in goat milk compared to cow milk accounts for the hypoallergenicity of goat milk (Juàrez and Ramos, 1986; Park, 1994), where lack

of α_{S1} -casein in goat milk is caused by the high degree of genetic polymorphism among goats (Bevilacqua et al., 2001; Tziboula-Clarke, 2003).

Comparing Holstein cow milk with Japanese-Saenen goat milk by SDS-PAGE, Tomotake et al. (2006) reported that caprine and bovine milk contained 3.9% and 33.7% α_{S1} -casein, respectively. However, SDS-PAGE could not identify α - and β -caseins variants such as α_{S0} -, β_{A1} -, and β_{A2} -casein in cow milk and β_1 -, and β_2 -casein in goat milk. As shown before (Jin and Park, 1996; Park, 2006), they identified the caprine casein had two major bands of β -casein and α_{S2} -casein, while the bovine casein showed the presence of two major bands of α_{S1} -casein and β -casein.

Capillary electrophoresis (CE) has been used to separate and analyze casein and whey protein fractions especially in the mixtures of cow, ewe, and goat milk (Molina et al., 1999) and their corresponding cheeses (Cattaneo et al., 1996; Molina et al., 2000). β_1 - and β_2 -casein were found as the major β -caseins in goat milk (García-Ruiz et al., 2000), and α_{S0} -, α_{S1} -, β_{A1} -, and β_{A2} -caseins were identified in cow milk (Miralles et al., 2000). However, limited information has been available for differences in casein protein

* Corresponding author. Tel.: +1 478 827 3089; fax: +1 478 825 6376.
E-mail address: parkyfvsu.edu (Y.W. Park).

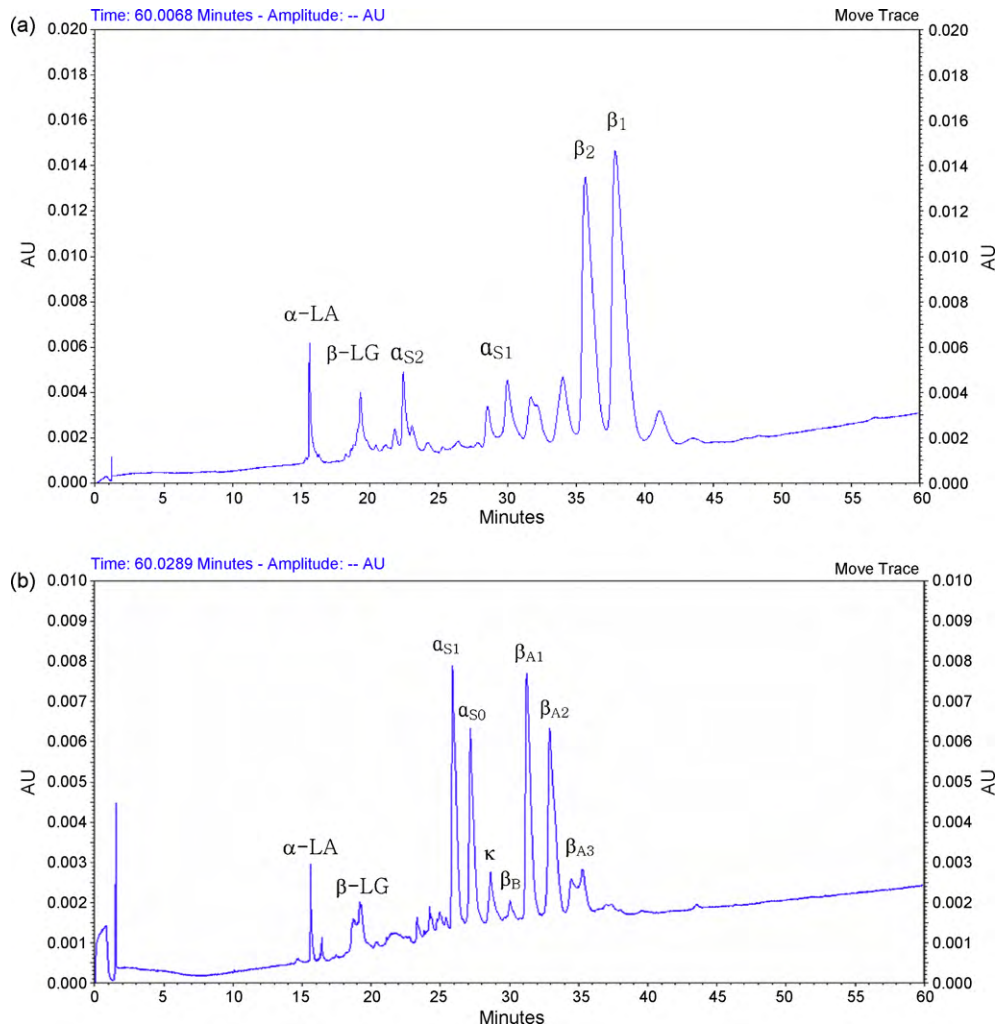


Fig. 1. Typical electropherograms of goat milk proteins (a) and cow milk proteins (b).

fractions between cow and goat milks quantitated by capillary CE analysis. Therefore, the objective of this study was to characterize the differences in α - and β -casein fractions of Korean-Saanen goats and Holstein cow milks, in conjunction with somatic cell counts testing.

2. Materials and methods

2.1. Preparation of milk samples

Goat milk samples were collected for 4 different times from bulk tanks of 10 different dairy farms located at Okcheon, Kang-Won province, South Korea. The number of goats in the 10 farms ranged from 100 to 500 milking Saanen goats. Holstein cow milk was also obtained 4 different times from the bulk tank of an experimental farm in the National Institute of Animal Science (NIAS), Suwon, Korea. Milk samples were transported under refrigeration to the analytical laboratories of NIAS and Institute of Livestock and Veterinary Research (Cheongwon, Chungeongbuk Province, Korea).

2.2. Analysis of somatic cell counts

Somatic cell counts (SCC) of all experimental goat milk and Holstein cow milk samples were assayed at the Institute of Livestock and Veterinary Research (Cheongwon, Korea) using Somacount 500 (Bent-

ley Instruments, Chaska, MN, USA). The Somacount 500 can analyze 500 samples/hr for somatic cell counts, where 3.5 mL milk sample, 0.0083 mg ethidium bromide, 3 mL manufacturer's test buffer and 500 mL/h of 2% PBS solution are required for one milk sample analysis.

2.3. Preparation of chemicals

Sodium hydroxide (analytical grade) was purchased from Junsei Chemical Co. (Tokyo, Japan). Hydrochloric acid, phosphoric acid (analytical grade), hydroxylpropyl methyl cellulose (HPMC), dithiothreitol (DTT), urea, and casein standards were purchased from Sigma (St. Louis, MS, U.S.A.). Trisodium citrate dihydrate was obtained from Merck (Darmstadt, Germany). All reagents were prepared with double deionized water using the Milli-Q system (Millipore Corp., Bedford, MA, U.S.A.).

The reduction buffer of capillary electrophoresis was prepared by dissolving 73 mg of trisodium citrate dehydrate and 38 mg of DL-dithiothreitol (DTT) in 37.5 mL of 8 M urea. The pH was adjusted to 8.0 with dilute sodium hydroxide solution in a 50 mL volumetric flask and made up to the volume with deionized water. The running buffer (50 mM) was prepared by mixing 14.7 M H_3PO_4 (847 μ L) and 0.05% HPMC with 6 M of urea solution (250 mL). Buffers were filtered through a 0.22- μ m Millex GV filter (Millipore Corp., Bedford, MA, U.S.A.).

2.4. Basic milk composition analysis

Milk fat, total protein and lactose contents were analyzed by a near infrared spectroscopy in the mid infrared region with a Milk-O-

Table 1Profiles of α s- caseins and β -caseins compositions of goat and cow milks determined by CE.^a

Farm no.	α_{S2}	α_{S1}	β_2	β_1	α_S^b	β^c	α_S/β
1	10.0 ± 0.27	4.9 ± 0.16	19.0 ± 0.84	40.4 ± 0.33	14.9 ± 0.35	59.3 ± 1.01	0.25 ± 0.01
2	8.2 ± 0.88	3.4 ± 1.58	19.1 ± 1.21	38.9 ± 1.78	11.6 ± 1.44	58.0 ± 2.97	0.20 ± 0.03
3	9.0 ± 0.09	7.4 ± 0.64	16.0 ± 0.72	34.9 ± 0.49	16.5 ± 0.73	50.9 ± 0.23	0.32 ± 0.01
4	8.4 ± 0.53	5.6 ± 0.29	15.5 ± 0.67	35.5 ± 0.83	14.0 ± 0.24	51.0 ± 1.39	0.27 ± 0.01
5	6.2 ± 0.30	5.3 ± 0.06	15.1 ± 0.08	35.1 ± 0.23	11.5 ± 0.36	50.2 ± 0.30	0.23 ± 0.01
6	5.6 ± 0.18	6.8 ± 0.18	33.3 ± 0.89	24.4 ± 0.97	12.4 ± 0.34	57.7 ± 0.32	0.21 ± 0.01
7	5.3 ± 0.69	9.1 ± 0.35	30.2 ± 0.42	22.3 ± 0.87	14.4 ± 1.03	52.5 ± 1.28	0.27 ± 0.03
8	5.0 ± 0.46	8.8 ± 0.46	27.5 ± 0.50	24.9 ± 0.83	13.8 ± 0.73	52.4 ± 0.88	0.26 ± 0.02
9	7.8 ± 3.48	4.5 ± 1.40	27.0 ± 1.53	27.0 ± 0.43	12.3 ± 2.07	54.0 ± 1.10	0.23 ± 0.04
10	5.6 ± 0.31	7.0 ± 0.15	24.5 ± 0.61	27.9 ± 0.20	12.6 ± 0.46	52.4 ± 0.81	0.24 ± 0.01
Ave.	7.4 ± 1.94	6.0 ± 2.00	22.3 ± 6.24	32.3 ± 6.67	13.3 ± 1.69	54.7 ± 3.65	0.24 ± 0.04
Max	10.3	9.3	34.3	40.9	17.0	61.0	0.33
Min	4.5	2.4	15.1	21.7	10.1	49.6	0.17
	α_{S1}	α_{S0}	β_{A1}	β_{A2}	α_S^d	β^e	α_S/β
Cow milk	19.6 ± 1.44	14.2 ± 0.41	22.0 ± 0.98	19.9 ± 1.19	33.8 ± 1.09	41.9 ± 0.41	0.81 ± 0.02

^a All values are mean ± standard deviation (SD), and all observations are average of 4 determinations.^b $\alpha_{S2} + \alpha_{S1}$.^c $\beta_2 + \beta_1$.^d $\alpha_{S1} + \alpha_{S0}$.^e $\beta_{A1} + \beta_{A2}$.

Scan instrument (N. Foss-Electric A/C, Hillerød, Denmark) using AOAC procedure (1990; No. 972.16).

groups of goat milk samples on the basis of somatic cell counts (1.5 million cells/mL).

2.5. Capillary electrophoresis analysis

Capillary electrophoresis (CE) was performed according to the modified methods described by Revilla et al. (2005) and De Jong et al. (1993) using a P/ACE™ system MDQ equipped with a DAD detector, temperature-controlled capillary compartment, and autosampler (Beckman Instruments, Fullerton, CA, U.S.A.). A 0.5 mL milk sample was diluted with 2.5 mL of the reduction buffer and incubated for 1 h at room temperature. For casein standards, 0.2 g each of α - and β -CN (Sigma Chemical Co.) was dissolved in 10 mL Milli-Q water, diluted with 2.5 mL reduction buffer and treated similar to milk sample. Upon incubation, the resulting clear solution was used for CE analysis. Separation of casein fractions were performed using a fused-silica capillary column (eCap™, Beckman) (60 cm × 50 μ m i.d.; 50 cm to the detector window) according to the corresponding peaks of α_S - and β -casein standards. Sample solutions were injected for 5 s at 0.5 psi. The separation was carried out at a constant voltage of 25 kV and at 20 °C. Peak detection was performed at the wavelength of 214 nm. Before each sample injection, the capillary was washed with 0.1 M NaOH for 5 min, deionized distilled water for 5 min, followed by 1 M HCl for 5 min, and then equilibrated with the running buffer for 5 min.

2.6. Statistical analysis

All experimental data were statistically analyzed by the SAS Enterprise Guide (SAS EG, Cary, NC, USA, 2006). The student's *t*-test was performed to compare the differences in levels of casein fractions between goat and cow milks, as well as differences in casein compositions between the two

3. Results and discussion

The characteristics of CE electropherogram of goat milk proteins are compared with those of cow milk counterparts as shown in Fig. 1. Different caseins were identified on the basis of their electrophoretic migration times with reference to the CE data reported previously (Garcia-Ruiz et al., 2000). The α_{S2} -CN of the experimental Saanen goat milk showed three peaks, and the next two peaks were marked as α_{S1} -CN. Genetic variants A, B, and C of α_{S2} -CN were found (Recio et al., 1997) and allelic variants of α_{S1} -CN was associated with α_{S1} -CN content (Grosclaude et al., 1987). The fastest electrophoretic component of goat casein (in alkaline systems) represents a much smaller proportion of total casein than the α_{S1} -CN in bovine casein (Parkash and Jenness, 1968). Gel electrophoretic patterns indicate that the total amount of α_S -CN is much less than that of the β -CNs (Jin and Park, 1996), while attempts to quantification by gel patterns have given conflicting results. CE has emerged as a powerful analytical tool that has several advantages over HPLC and slab gel electrophoresis (Blanc et al., 1997). CE offers very high resolving power,

Table 2Casein compositions of the goat milk grouped by somatic cell counts.^a

SCC	α_{S2}	α_{S1}	β_2	β_1	α_S^b	β^c	α_S/β
<1,500,000 ^d	7.01 ± 1.84	4.94 ± 2.05	25.55 ± 5.80	31.06 ± 6.95	11.94 ± 1.11	56.61 ± 2.94	0.21 ± 0.03
>1,500,000 ^e	7.58 ± 2.02	6.62 ± 1.74 [*]	20.28 ± 5.76 [*]	33.14 ± 6.57	14.20 ± 1.39 ^{**}	53.42 ± 3.58 [*]	0.27 ± 0.03 ^{**}

^a All values are mean ± SD.^b $\alpha_{S2} + \alpha_{S1}$.^c $\beta_2 + \beta_1$.^d Data from 4 farms; 4 replicates of bulk tank samples from each farm (16 observations).^e Data from 6 farms; 4 replicates of bulk tank samples from each farm (24 observations).^{*} Significant at *P* < 0.05.^{**} Significant at *P* < 0.01.

low solvent consumption, rapid analysis, produces quantitative results, and is automated (Belinda, 1997). Because of these advantages, CE may be well suited for quality control purposes by the dairy industry and by regulatory agencies.

Table 1 shows the values of α s-CN and β -CNs and their ratios in the goat of 10 different goat farms and cow milk samples from NIAS dairy cattle farm. The α s₁-CN content of the goat milk samples was varied from 3.4% to 9.1% of total proteins in our Korean-Saenen goat milk. The β -CNs are quantitatively the major protein components of goat milk, and isolated two goat β -CNs representing the most prominent electrophoretic bands in goat casein (Juárez and Ramos, 1986; Jin and Park, 1996; Park, 2006). The β -CNs content in the present study was calculated by adding the β ₁-CN and the β ₂-CN content, which varied from 49.6% to 61.0% of total proteins. The average α s-CN to β -CN ratio of the raw Korean-Saenen goat milk samples was 0.24 ± 0.04 , and ranged from 0.17 to 0.33 depending on different farms. These results suggest that CE analysis could be used as a quick and efficient method of detecting possible fraudulent addition of cow milk to goat milk.

Caseins contents in the goat milk samples showed significant difference when grouped on the basis of the levels of somatic cell counts. Higher SCC (more than 1.5 million cells/mL) goat milk showed higher α s-CN contents and lower β -CNs contents than those below 1.5 million SCC (Table 2), and resulting in higher α s-CN to β -CN ratio for the high SCC goat milk. Six out of 10 goat farms showed over 1.5 million/mL SCC, and also higher SCC milk samples showed higher protein and fat concentrations, which is in agreement with the findings of Leitner et al. (2004). However, studies with sheep milk are contradictory. No significant decrease in casein content was associated with SCC (Pirisi et al., 2000), while a decrease in casein content occurred when SCC increased (Jaeggi et al., 2003).

The present study indicates that α s-CN to β -CN ratio in higher SCC goat milk increased by the decrease in relative amounts of β -CNs. These compositional changes may alter the processing qualities of the goat milk in relation to manufactured products including goat milk cheeses, where milk having a higher α s₁-CN content would have a firmer curd formation during coagulation process of cheese-making (Remeuf, 1992).

4. Conclusions

Quantitative determination of casein fractions in goat and cow milk by capillary electrophoresis has revealed that there were significant differences in casein compositions between the Korean-Saenen goats and Holstein cow milks. The α s-CN to β -CN ratio of goat milk was much smaller than that of the cow milk, and was significantly increased by the decrease of β ₂-casein in the goat milk samples having more than 1.5 million somatic cell counts. Since α s₁-CN content is critical for gel formation during coagulation process, further studies are desirable to evaluate the effects of different casein contents on processing qualities of Korean-Saenen goat milk.

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